The North African Journal of Food and Nutrition Research: (2019)03;(06):180-85
https://doi.org/10.5281/zenodo.3358250

Original Article

OPEN ACCESS

eISSN: 2588-1582

Making Nutrition a Development Priority in Africa

Contents lists available at

Journal homepage: https://www.najfnr.org

Physical properties and chemical composition of three Ethiopian rice (*Oryza sativa* Linn.) varieties compared to tef [*Eragrostis tef (Zucc.)* Trotter] grain

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ARTICLE INFO

Article history:
Received 03 June 2019
Accepted 25 July 2019
Published 12 August 2019

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Access this article online

Quick Response Code

https://doi.org/10.5281/zenodo.3358250

Article edited by:
Dr. Oudir BOUSSOUM
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ABSTRACT

BACKGROUND: It is really crucial to explore physical properties and chemical composition of the locally cultivated cereal crops to enhance their utilization in various recipes. Moreover, that will fill knowledge gaps in this field and provides advantages for both; producers and consumers who still believe that imported cereals are superior to the locals. **AIMS**: This study was intended to investigate the physical properties and chemical composition of the three rice varieties *(Edeget, Nerica-4, and X-jigna)* in comparison to brown tef which are grown in Ethiopia. The evaluation included determination of thousand kernel weight (TKW), hectoliter weight (HLW), moisture, ash, crude fiber, crude fat, crude protein, carbohydrate, Fe, Zn, Ca, and phytic acid contents. **DATA ANALYSIS**: The data analysis was conducted using SPSS Version-22. Duncan's multiple range test was used for the mean comparison at $p<0.05$. **RESULTS**: The results revealed that the rice cultivar Edeget showed a higher TKW (39.20 g) than other varieties while brown tef had a TKW of 0.36 g. X-jigna displayed a higher HLW (63.70 kg/hL) than other varieties while the brown tef had 84.48 kg/hL. The ash, crude fiber and fat contents of the brown tef were higher than all the three rice varieties. Nerica-4 had better protein content (9.61%) than other rice varieties and brown tef had a protein content of 9.58%. The iron content of the three rice varieties was virtually null while the brown tef had shown a higher iron content (17.18 mg/100g). Nerica-4 had shown a higher zinc content (3.62 mg/100g) while the brown tef had got higher calcium (91.90 mg/100g) and phytic acid (5 mg/g). **CONCLUSIONS**: The study revealed that the physical properties of the three rice varieties were significantly different regardless of the environmental influences.

KEYWORDS: Physical properties, chemical composition, varieties, rice, tef

1. INTRODUCTION

Rice (*Oryza sativa* Linn.) is the second most important cereal grain next to wheat and imparts an essential role in human nutrition especially for developing countries. Rice, constitutes a popular gluten-free source of carbohydrates, and non-allergenic to celiac patients, contains about 7.5% high-quality protein [1]. Moreover, the grain represents a source of income for a large number of people throughout the world. In Ethiopia, rice cultivation has no long history and still, it is not a widely cultivated crop. However, the crop has been used in different forms even before its cultivation was introduced in some parts of the country. Furthermore, it was one of the imported cereal crops in Ethiopia. Rice is utilized to prepare *injera* (alone or by mixing with other cereals such as tef and sorghum), bread, cooked rice and *kinche* (splatted cooked rice). Moreover, it is used for brewing local alcoholic drinks such as *farsso* and *areke*. However, rice over-consumption results in constipation particularly in children due to its lower fiber content.

Additionally, mineral and fat content of rice is reduced compared with other cereals. Therefore, blending rice with other grain such as tef can ameliorate its compositions.

Contrarily, rice grain has come to be one of the important raw materials in the food processing industry because of its inimitable characteristics such as eye-catching white color, lower allergenic, and assimilation [1]. Moreover, rice shows the best expansion property, due to its higher starch composition, and being therefore, appropriate for processing a variety of food products [2].

Tef [*Eragrostis tef (Zucc.)* Trotter] is a stable and fascinating crop which is minute in size, packed with nutrition that supplies the major calorie for the most majorities of Ethiopians [3]. The tef kernel is too small with a mean length and width of 1 to 1.20 and 0.59 to 0.75 mm, respectively [4,5], to isolate the germ from the bran, as a result, the germ and the entire seed is consumed. This is a basic secret behind the greater fiber content and enhanced nutritional benefaction of the tef grain.

The tef grain has an equivalent nutritional composition with the main crops like oats, wheat, barley and rice and superior in some contents [6]. Tef grain has crude protein (11%), carbohydrate (73%), crude fiber (3%), fat (2.5%) and ash (2.8%). The thousand kernel weight of the grain is also in the range of 0.19 to 0.42 g [3].

Nowadays, the tef grain is consumed as a primary food for the over millions of Horn African populations [7]. Basically, the grain is utilized for the preparation of *injera*, a pancake-like fermented flatbread [8] mainly consumed in Ethiopian by almost all age groups. Tef grain, that is gluten-free in nature [6] and has higher iron content as compared to other cereal crops and some legumes [3] constitutes the best source of vitamin B and minerals with a high amount in amino acid compared to other cereal crops like wheat and barley [3] which is comparable to eggs protein except for its lower lysine content [9]. In this paper, I attempted to present the physical properties and the chemical composition of three rice varieties in comparison to brown tef which are locally cultivated in Ethiopia. It was also aimed to escalate the utilization of the locally produced rice varieties in various product developments in combination with the tef to benefit from the combined chemical composition of the grains as well as the reduced cost of the product.

2. MATERIAL AND METHODS

2.1. Experimental materials

Three rice (*Oryza sativa* Linn.) varieties (Edeget, Nerica-4, and X-jigna) and brown tef [*Eragrostis tef (Zucc.)* Trotter] were obtained from Adet Agricultural Research Center (AARC), Bahirdar, and Debre Zeit Agricultural Research Center (DZARC), Debre Zeit, Ethiopia, respectively. The samples were manually cleaned and divided into two parts. One part was reserved for the study of physical properties, while the second one was milled and sieved with 200 µm sieves to get a uniform flour size. All samples were kept in polyethylene bags at room temperature throughout the analysis period. All the samples used in this piece of work were cultivated in Ethiopia (harvest of 2016/17) and the whole experiments were conducted in a very reputable Haramaya University, Ethiopia.

2.2. Analytical methods

2.2.1. Grain physical properties

a. Thousand kernel weight (TKW)

TKW was determined as described in AACC [10]. Broken grains and foreign materials were handpicked from the sample. Thousand-grain kernels were counted by seed counter (Numigral II, chopin seed counter, France). The weight of 1000 grains (TKW) was expressed in relation to the dry matter.

b. Hectoliter weight (HLW)

HLW of the grain samples was analyzed as described in AACC [10] method 50 – 10. Dockage free grain sample was prepared and poured into a graduated measuring cylinder. Pouring of the grain sample took place from a height of 15 cm above the cylinder in a regular stream to reduce variation in the degree of packing in a series of trials. The mass of the grain was measured on a digital balance. Finally, the hectoliter weight of the grain sample was calculated and reported as kg/hL.

2.2.2. Proximate compositions

Moisture, ash, crude fat, and crude fiber contents of the samples were analyzed following AOAC [11]. The crude protein [CP (%) = N (%) * 6.25] was calculated after analyzing the nitrogen content by a micro-Kjeldahl method [10]. The total carbohydrate was calculated by differences [100 – (Moisture (%) + Crude ash (%) + Crude protein (%) + Crude fat (%) + Crude fiber (%))] based on Egan *et al.*, [12].

2.2.3. Mineral analysis

The mineral analysis was carried out using atomic absorption spectrophotometer (Model: 210 VGP spectrophotometer, Bulk Scientific, East Norwalk, CT, USA) after wet digestion of about 3 g sample using air-acetylene as a source of energy for atomization [10]. For iron, zinc and calcium determination absorbance were measured at 248.3 nm, 213.8 nm, and 422.7 nm, respectively. Thus, the mineral

contents of the samples were estimated from their respective standard calibration curves.

2.2.4. Phytic acid determination

Phytic acid was calculated following the extraction of 0.25g sample in 12.5 mL of 3% trichloroacetic acid, sleet of phytate in the form of ferric phytate by the addition of 4mL of $FeCl_3$ (2 mg/mL) [13] succeeded by phytate phosphorus (Ph-P) analysis [14]. Thus, phytate = P × 3.55 [13].

2.3. Data analysis

All parameters were done in triplicates. The data were examined by analysis of variance (ANOVA) employing IBM SPSS Statistics for Windows, Version 22. Armonk, NY: IBM Corp, USA. The means were compared at $p<0.05$ using Duncan's Multiple Range Test (DMRT).

3. RESULTS AND DISCUSSION

3.1. Thousand kernel weight (TKW) and hectoliter weight (HLW)

The TKW for each rice varieties and tef grain are shown in Table 1. The TKW of the three rice varieties (Edeget, Nerica-4, and X-jigna), were 39.20, 33.57 and 36.63 g, respectively and that of tef was 0.36 g. The variation in TKW among the rice varieties and the tef grain was statistically significant ($p<0.05$). Rice variety, Edeget has got higher TKW while tef has recorded the smallest TKW. This result showed that the TKW of the grain is clearly a result of the grain size. The values of TKW for rice varieties were in the range of 12.5 to 42.7 g as reported by Yan *et al.,* [15]. The TKW of tef was in the range of 0.19 to 0.42 g which is similar to what was reported by Bultosa [3].

The HLW of Edeget, Nerica-4, and X-jigna rice varieties were 63.57, 59.70 and 63.70 kg/hL, respectively. In previous reports, the HLW of rice was measured in the range of 46.6 - 53.8 kg/hL and this value could reach up to 51.77 - 54.57 kg/hL depending on the selected irrigation method. The HLW of tef was 84.48 Kg/hL which is in agreement with the value reported by Bultosa [3]. Incredibly the tef grain has shown a significantly higher HLW than other rice varieties. This is basically due to the size, shape and packing nature of the grain.

3.2. Proximate compositions

The proximate composition of three rice varieties and tef grain are summarized in Table 1. The moisture contents of Edeget, Nerica-4, and X-jigna rice varieties were 10.10, 10.26 and 10.09%, respectively which is less than 12.30% reported by Hema *et al.* [16]. The moisture content of tef was 10.41%. The moisture content found in tef flour agreed with the mean (10.26%) reported by Bultosa and Taylor [4]. The ash contents of Edeget, Nerica-4, and X-jigna rice varieties were 0.73, 0.64 and 0.94%, respectively which is closer to the mean (0.62%) obtained by Hema *et al.,* [16] and the ash content of tef was 2.94% which was in the range of 1.99% to 3.16% [3]. The ash content of tef grain is higher than rice. Because the grain is too small making difficult to separate the bran from the germ and its bran is proportionally large and the bulk of the flour consists of the bran and germ [3].

The crude fiber content of Edeget, Nerica-4, and X-jigna rice varieties were 0.11, 0.17 and 0.27%, respectively which is closer to the mean value (0.21%) obtained by Sotelo *et. al.,* [17]. Milling of rice to white grain rice generally decreases the fiber content of rice. The crude fiber content of tef was 2.04% which is in agreement with (2.0 to 3.5%) as reported in Bultosa [3]. The tef grain has a better fiber content than most cereal crops due to the smaller size of the grain to remove the bran and the bran is comparably giant [4]. The crude fat content of Edeget, Nerica-4, and X-jigna rice varieties were 1.89, 1.61 and 1.78%, respectively. These results are in the range of 0.9 to 1.97% [18]. Milling of rice removes the outer layer (aleurone layer) of the grain where most of the fats are concentrated [19]. The crude fat content of tef grain was 3.07% which is similar (2.0 to 3.1%) to the reports of Bultosa [3].

The protein content of Edeget, Nerica-4, and X-jigna rice varieties were 8.42, 9.61 and 8.24%, respectively. The obtained results agree with those of Ebuehi & Oyewole [20] (8.3%). The crude protein content of the tef grain was 9.58% which is in the range of 9.4 to 13.3% [3]. Though the rice crop is considered as the lowest protein source than other cereals such as wheat, corn, and barley, its overall protein utilization is the highest [18]. However, the rice varieties used in this study has shown better protein content than previously reported. Rice protein is also known for its lower allergenicity than other grains and legumes protein. Thus, mainly it is used as a source of protein in infant formulas and for celiac patients [1].

The protein in tef is similar to other grains. However, tef grain contains slightly higher methionine, phenylalanine, and histidine than most other grains and lower serine and glycine. The essential amino acid lysine is higher in tef than in most other grains, except rice and oats. The balance of essential amino acids in tef is equivalent to egg protein apart from its lower lysine content [9].

In this study, rice samples contained high carbohydrates ranging from 77.71 to 78.74% that agree with Edeogu *et al.,* [21] and lower in moisture, ash, fiber and fat contents than tef. The total carbohydrate content of tef was 71.35%. The total carbohydrate for tef grain was in close agreement with the reported value of Bultosa [3] (73%).

Table 1: The TKW, HLW and proximate composition of three rice varieties and tef grain

Grains	TKW (g)	HLW (Kg/hL)	Moisture (%)	Ash (%)	Crude fiber (%)	Crude fat (%)	Crude protein (%)	Carbohydrate (%)
Edeget	39.20±0.66^{a}	63.57±0.37^{b}	10.10±0.09^{bc}	0.73±0.04^{c}	0.11±0.00^{d}	1.89±0.02^{b}	8.42±0.17^{b}	78.74±0.27^{a}
Nerica-4	33.57±0.49^{c}	59.70±0.29^{c}	10.26±0.13^{ab}	0.64±0.04^{d}	0.17±0.03^{c}	1.61±0.03^{d}	9.61±0.14^{a}	77.71±0.29^{b}
X-jigna	36.63±0.32^{b}	63.70±0.67^{b}	10.09±0.05^{c}	0.94±0.04^{b}	0.27±0.03^{b}	1.78±0.07^{c}	8.24±0.07 ^{b}	78.69±0.20^{a}
Tef	0.36±0.02^{d}	84.48±1.04^{a}	10.41±0.05^{a}	2.94±0.01^{a}	2.04±0.02^{a}	3.07±0.01^{a}	9.58±0.16^{a}	71.35±0.14^{c}

Values are in Mean ± SD on a dry matter basis. Means within a column with different superscripts are significantly different at P<0.05. Where: TKW is thousand kernel weight and HLW is hectoliter weight.

The carbohydrate of tef grain is virtually starch that displays slow retrogradation property making the grain one of the best alternatives for diabetes patients [4]. Moreover, the gluten-free nature of tef and rice, as well as the smaller size of their starch granules, make the crops to be widely utilized in various forms. Also, imparts to the highly escalating prices of the grains.

3.3. Mineral and phytic acid contents

The mineral and phytic acid content, analyzed for the three rice varieties and brown tef grains, are given in Table 2. The analysis showed that rice had insignificant (p>0.05) iron content (virtually zero). Rice actually has iron, but only in the seed coat which is easily removed during dehulling and/or milling. The iron content of brown tef was 17.18 mg/100g which were found to be less than 37.70 mg/100g [22]; this may be due to contamination and varietal difference.

Table 2: Minerals and phytic acid contents of three rice varieties and tef grain

Grains	Fe (mg/100g)	Zn (mg/100g)	Ca (mg/100g)	Phytic acid (mg/g)
Edeget	0.00^{b}	3.08±0.00^{c}	10.72±0.81^{b}	1.45±0.17^{d}
Nerica-4	0.00^{b}	3.62±0.03^{a}	8.34±0.44^{c}	2.70±0.07^{b}
X-jigna	0.00^{b}	2.71±0.01^{d}	9.91±0.48^{b}	2.43±0.10^{c}
Tef	17.18±0.07^{a}	3.45±0.01^{b}	91.90±0.48^{a}	5.00±0.03^{a}

Values are in Mean ± SD on a dry matter basis. Means within a column with the same superscripts are not significantly different at p>0.05. Where: Fe is iron, Zn is zinc and Ca is calcium.

The zinc content of Edeget, Nerica-4, and X-jigna rice varieties were 3.08, 3.62 and 2.71 mg/100g, respectively which is in a close agreement with Sotelo *et al.,* [17] (1.6 – 3.1 mg/100g). In the current study, tef grain contained 3.45 mg/100g zinc and was higher than 2.86 mg/100g as found by Abebe *et al.*, [22]. The calcium content of Edeget, Nerica-4, and X-jigna were 10.72, 8.34 and 9.91 mg/100g, respectively which was in the range of 3 – 11 mg/100g [23] and the calcium content of tef was 91.90 mg/100g. The calcium content of tef grain was lower than 124.00 mg/100g, reported by Abebe *et al.*, [22]. The phytic acid content of Edeget, Nerica-4, and X-jigna were 1.45, 2.70 and 2.43 mg/g, respectively which is slightly higher than reported by Kennedy *et al.,* [24]. The phytic acid content of grains may vary due to grain varieties, climatic condition, as well as pre and post-processing conditions [25].

The mineral contents of crops may vary along with location and from country to country. For instance, the Fe content of brown rice is 0.022 mg/g in India and 0.012 mg/g in Vietnam [26]. Similarly, Zn shows some disparity about 0.20 mg/100g [27,28].

The minerals' distribution in rice kernels is not uniform. The bran contains about 50% of the minerals and embryo contains 10%. However, both the bran and embryo will be discarded during white rice production. Ash content is a good indicator of mineral compositions and white rice comprises around 28% of the brown rice [29]. Brown rice has about 120 mg/100g phytic acids and this composition varies with location and variety [24,30]. Phytic acid influences the solubility and bioavailability of minerals and proteins by forming a complex with them. Furthermore, it is observed to be one of the considerable factors for iron deficiency anemia due to low bioavailability of iron even in a high iron intake [30].

Phytic acid, well-thought-out as one of the antioxidants in cereals [31], was suggested as a cure for colon cancer [32] and could reduce the noxiousness of certain heavy metals due to its strong chelating ability with metals [33]. The phytic acid content of tef was 5.00 mg/g which is lower than 8.42 mg/g as reported by Abebe *et al.*, [22]. This may be due to grain variety differences.

4. CONCLUSION

The present study showed that the physical properties of the three rice varieties were significantly different regardless of the environmental influences. Because the three rice cultivars were collected from the same site under the same environmental conditions and the same treatments. The three rice cultivars had a higher TKW than brown tef. The brown tef had a higher HLW, due to a high compaction rate mainly because of the smaller grain size. Furthermore, brown tef was found superior in ash, crude fiber and fat content over the three rice varieties. Fe and Ca contents were the highest in brown tef, when Zn was higher

in rice cultivar Nerica-4 than brown tef. However, Brown tef showed a higher phytic acid content.

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Cite this article as: Legesse S. The physical properties and chemical composition of three Ethiopian rice (*Oryza sativa* Linn.) varieties compared to tef [*Eragrostis tef (Zucc.)* Trotter] grain. *Nor. Afr. J. Food Nutr. Res.* July - December (2019);03(06):180-85. https://doi.org/10.5281/zenodo.3358250

The North African Journal of Food and Nutrition Research: (2019)03;(06):186-94
https://doi.org/10.5281/zenodo.3497409

Original Article

OPEN ACCESS

Making Nutrition a Development Priority in Africa

eISSN: 2588-1582

Contents lists available at

Journal homepage: https://www.najfnr.org

Effect of traditional sun-drying and oven-drying on carotenoids and phenolic compounds of apricot (*Prunus armeniaca* L.)

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ARTICLE INFO

Article history:
Received 29 June 2019
Accepted 06 October 2019
Published 10 October 2019

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Access this article online

Quick Response Code

https://doi.org/10.5281/zenodo.3497409

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ABSTRACT

Background: The indubitable role of phytochemicals such as carotenoids and phenolic compounds in human health has prompted the researchers to study the factors affecting the stability and the availability of these compounds. **Aims**: This study investigates the effect of two drying processes; oven-drying (OD) and traditional sun-drying (TSD) on carotenoids and phenolic compounds of apricots. **Methods and Material**: OD was performed at 65°C, and TSD was performed by direct exposure of apricot to sunlight at daytime temperatures around 40°C and relative humidity between 25 and 35%, following an Algerian traditional method of drying. Carotenoids and phenolic compounds were extracted, and then total carotenoids (TC), total phenolic compounds (TPC), total flavonoids (TF) and total tannins (TT) were spectrophotometrically quantified. The free radical scavenging activity (FRSA) of the phenolic extracts was measured by the DPPH method. **Results**: Carotenoids and phenolic compounds were significantly affected by both drying methods. OD decreased TC and TT by 44% and 12%, respectively, and increased TPC and TF by 4%. TDS affected negatively all the measured components, where TC, TPC, TF, and TT decreased by 67%, 15%, 43%, and 36%, respectively. However, the highest FRSA was reported for the TSD apricots (40%) followed by OD apricots (36%), and fresh apricots (32%). **Conclusions**: The effect of drying on apricot antioxidants depends on the applied drying method and the studied component. The direct sunlight exposure and the duration of drying condemned TSD to be more harmful on carotenoids and phenolic compounds compared to OD, where carotenoids where more fragile during TSD. In addition, OD improved the content of phenolic compounds by improving their extractability. However, TSD apricots seem to be a better source of free radical scavenging compounds.

Keywords: Apricot, traditional sun-drying, oven-drying, carotenoids, phenolic compounds.

1. INTRODUCTION

Fruits are excellent sources of macro and micronutrients, particularly bioactive compounds such as vitamins and antioxidants. Nowadays, it's clear that antioxidants as phenolic compounds, carotenoids, and vitamin C impart numerous health benefits, where the prevention of some socially significant diseases (like cancer and cardiovascular diseases), has been associated with consumption of fresh fruits and vegetables [1,2]. Plant antioxidants are phytochemicals that can prevent the oxidation of a biological substrate. Thus, protecting food and tissues from damages that can be caused by free radicals [3]. Apricots are widely distributed fruits, due to their specific sweet flavor and color. Every year, more than 4.2 million tons are produced [4]. Furthermore, apricot constitutes one of the most cultivated fruits in the North African region, particularly in Algeria, which covers more than 6 % of the world's production [4]. Apricots provide significant health benefits because of their high content in antioxidants, primarily phenolic compounds and carotenoids [5,6]. Phenolics represent the predominant phytochemicals

present in apricots [5]. These compounds are a structurally diverse class of phytochemicals and they occur as plant secondary metabolites, they are defined by the presence of at least one aromatic ring bonded directly to one or more hydroxyl groups [2]. Along with their antioxidant activity, Phenolics showed several further biological characteristics such as antimicrobial, anti-inflammatory, and immuno-stimulatory activities [7]. Flavonoids are water-soluble phenolics that show strong antioxidant activities, they constitute the largest group of polyphenols, with more than 5000 identified compounds [8]. Carotenoids are a large family of lipophilic compounds that are responsible for the orange color of apricots; they play a significant role in light-harvesting and in protection against photodamage in plants [9]. Carotenoids have been found to exhibit important antioxidant activity and help in preventing chronic diseases such as cardiovascular disease and skin cancer [10]. Furthermore, they are referred to as provitamin A since they can be transformed *in vivo* to active vitamin A[9, 10].

Apricots are climacteric fruits that undergo fast maturation after harvesting, which considerably limits their period of storage. Thus, different preservation methods such as drying and canning are habitually applied to preserve the fruits. Drying is the most common method to preserve apricots and extend their availability [11]. The process reduces the moisture content of apricots to a degree that allows safe storage for a longer period [12]. However, several studies reported that the antioxidant content of fresh fruits can be affected by processing techniques, which can increase or decrease their content [8, 13, 14].

Sun-drying of fruits and vegetables is one of the oldest forms of food preservation methods. In Algeria, traditional sun-drying of apricot remains the most practiced method. The reason behind this is that sun-drying is a simple method, requiring low capital, simple equipment, and low energy input. The traditional process of drying, applied in Algeria, is different from that usually reported in the literature, where apricots are neither blanched, nor treated with sulfates to prevent browning. Instead, apricots are treated with salt as a preservative agent and then dried. This method provides to the dried apricots specific organoleptic properties, where the color of the product is brown to dark with a salty flavor. Thus, there is a lack of knowledge on the effect of this traditional procedure on apricot antioxidants. For this reason, the current work investigates the effects of traditional sun-drying (TSD) on apricot antioxidants, primarily polyphenols, flavonoids, tannins, and carotenoids. The effect of oven drying (OD) was also investigated for comparison.

2. MATERIAL AND METHODS

2.1. Plant materials

Fruits of *Prunus armeniaca* L. (cv. Louzi) were collected from the N'gaous region of Algeria at commercial maturity. Apricots were directly transported to the laboratory, rinsed with tap water and stored at 4°C until utilization.

2.2. Drying process

2.2.1. Oven-drying

Fruits were dried in a ventilated laboratory oven (Memmert ULE 600, Germany) at a drying temperature of 65°C (Figure 1). The temperature of 65°C constitutes the average temperature used by food technologists to dry apricot [15-18]. Samples of fresh apricots were halved, pitted, and then placed in the oven, on the steel sieved trays, which were designed to increase air passage from both surfaces. Before starting the drying process, the oven was run for 30 min to obtain steady-state conditions. The suitable dryness level (moisture around 25 %, according to *CODEX STAN 130-1981*) was reached in 14 to 15 hours. Dried apricots were placed into polyethylene bags and stored at 4°C until subsequent analysis.

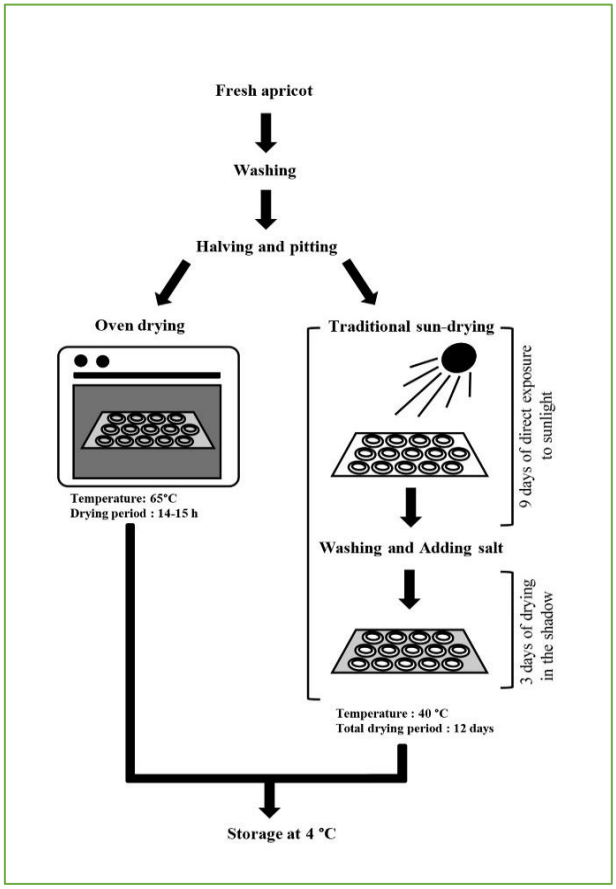

Figure 1: Simple schematization of the drying processes

2.2.2. Traditional sun-drying

Fresh apricots were halved, pitted, divided on a metal plate (covered with a cloth) and then dried by direct exposure to sunlight, with an overall maximum daytime air temperature of around 40°C for nine days (starting from mid-June). After that, dried apricots were washed, treated with salt by pulverization, and then, dried for three other days in the shadow to evaporate the residual water from the washing step (Figure 1). Dried apricots were placed into polyethylene bags and stored at 4 °C until subsequent analysis. During the drying process, the relative humidity of the air was between 25 and 35%, the days were sunny with no precipitation. This traditional sun-drying method is a common process applied by the farmers and the families in several regions in Algeria, aiming to preserve the excess of production and make apricots available for longer periods.

2.3. Chemical properties

Aiming to facilitate the extractability of apricot antioxidants and to standardize the analysis conditions for all samples, dried apricots were rehydrated for 24 h at room temperature. The exact and identical amount of water lost during the drying process was added during the rehydration (to reach the same water content as the fresh fruit), while the fresh samples were directly analyzed [19]. Before analysis samples were homogenized for two minutes using a Hand Blender Beaker. The following analyses were performed on the obtained purees: moisture content (MC) and dry-matter content (DM). They were measured (%) in a vacuum oven for 3 h at 105 °C (NF V 05-108, 1970) (for fresh, dried and rehydrated fruit), pH, measured using a digital pH meter (NF V 05-108, 1970). Acidity was determined as gram of citric acid per 100 g of samples by titration with 0.1 N sodium hydroxide to endpoint (pH 8.3) (NF V 05-101,1974). Ash content (%) was obtained using a muffle furnace at 550 °C for 5 hours (NF V 05-113,1972).

2.4. Measurement of total carotenoids

Total carotenoids were extracted according to the method of Rodriguez-Amaya [20] with optimization. Five grams of sample were extracted with 100 mL of methanol/petroleum ether (1:9, v/v) by using a high-speed homogenizer, and the mixture was transferred to a separating funnel. The petroleum ether layer was filtrated through sodium sulfate, transferred to a volumetric flask, and then the volume was completed to 100 mL with petroleum ether. Finally, the total carotenoid content was measured at 450 nm using a Shimadzu 1600- UV spectrophotometer. The results were expressed in mg β-carotene equivalent (β-CE/100 g DM).

2.5. Phenolic compounds analysis

2.5.1. Phenolic extract preparation

Phenolic compounds were extracted using the method described by Ali *et al.* [21]. Five grams of fruit puree was taken from the homogenate and diluted to 30 mL with 80% methanol and clarified by centrifugation (SEGMA 3-30K) at 10,000 × g for 15 min. The extract was filtered through a Whatman no. 1 filter paper.

2.5.2. Total *phenolic compounds* measurement

Total phenolic compounds (TPC) were measured by using the Folin–Ciocalteu assay as described by Singleton *et al.* [22] with minor modification. The crude phenolic extract, 0.5 mL was first diluted to 5 mL with 80% methanol, then 0.5 mL of 2 N Folin–Ciocalteu reagent and 0.5 mL of 20% sodium carbonate solution were added. The mixture was then allowed to stand for 60 min at room temperature and the absorbance was measured at 765 nm using a Spectrophotometer. Total phenolics were estimated by calibration curve prepared with concentrations of 0.01-0.25 mg/mL of gallic acid. The results were expressed in mg gallic acid equivalent (GAE) / 100 g DM.

2.5.3. Total flavonoids measurement

Total flavonoids (TF) were determined using the colorimetric method described by Bahorun *et al.* [23]. From the crude phenolic extract, 1 mL was mixed with 1 mL of a 2% $AlCl_3$-$6H_2O$ solution. After 10 min, the absorbance was measured immediately at 430 nm using a Spectrophotometer. The results were expressed as mg quercetin equivalent (QE) /100 g DM, according to a calibration curve prepared with concentrations of 1-40 µg/mL of quercetin.

2.5.4. Total tannins measurement

The estimation of the total tannins (TT) content was carried out by the method described by Hagerman & Butler [24]. 1 mL of the phenolic extract was mixed with 2 mL of bovine serum albumin solution (1 mg/mL) prepared in 200 mM acetate buffer, pH 4.9. After immediate stirring and incubation for 24 hours at 4 °C, the mixture was centrifuged for 15 min at 4000 rpm. The supernatant was discarded and the pellet was recovered and washed with 200 mM acetate buffer, pH 4.9. The resulting precipitate was dissolved in 4 mL of sodium-dodecyl-sulfate/*Tri-ethanol-amine* (1:5, w/v) solution, pH 9.5, and then 1 ml of the ferric chloride solution (100 mM HCl, 10 mM $FeCl_3$) was added. After incubation for 15 min, the absorbance was read at 510 nm on a Spectrophotometer. The amount of tannins was calculated by a calibration curve prepared with tannic acid (0.1-1.25 mg/ml). The results are expressed in mg tannic acid equivalent (TAE)/100 g DM.

2.5.5. Free radical scavenging activity measurement

Free radical scavenging activity (FRSA) was measured using DPPH (2,2-diphenyl-l-picrylhydrazyl) free radical according to the protocol described by Kuskoski *et al.* [25]. 0.1 ml of crude phenolic extract was taken in the test tube and 3.9 mL of 100 µM DPPH (2,2-diphenyl-l-picrylhydrazyl) solution was added, then the mixture was shaken vigorously and incubated for 30 minutes at room temperature. Absorbance was measured at 517 nm using a Spectrophotometer. The DPPH solution, freshly prepared with 80% methanol, gives an absorbance of 1.1 at 517 nm. Radical scavenging activity was calculated as % inhibition of DPPH radical using formula (01):

$$\%\text{Inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad \ldots\ldots\ldots (01)$$

$A_{control}$: Absorbance of the control reaction (blank with methanol and DPPH solution).

A_{sample} : Absorbance of the sample reaction (phenolic extract with methanol and DPPH solution).

2.6. Statistical analysis

All analyses were carried out in triplicate and the experimental data were reported as means ± standard deviation (SD). The data were subjected to an analysis of variance (one-way ANOVA). The significant difference was determined by Tukey's multiple range test ($p \leq 0.05$) using XL-STAT software Version 2009.

3. RESULTS

The aim of the current study is to investigate the effect of traditional sun-drying (TSD) and oven drying (OD) on apricot antioxidants (carotenoids and phenolic compounds). Prior to the measurements on antioxidants, fresh and dried apricot were first assayed for humidity, ash content, pH and acidity. The results are shown in table 1.

Table 1: Chemical properties of fresh and dried apricots

Drying process	MC (%)	DM (%)	Ash (%)	pH	TA (%)
FA	85.24 ± 0.23	14.76 ± 0.23	0.734 ± 0.08	3.94 ± 0.07	0.59 ± 0.06
TSD	21.05 ± 0.56	79.95 ± 0.56	4.26 ± 0.18	4.03 ± 0.17	3,62 ± 0,13
OD	26.06 ± 0.74	73.94 ± 0.74	2.82 ± 0.08	4.16 ± 0.05	2.87 ± 0.08

FA, fresh apricot; TSD, traditional sun drying; OD, oven drying; MC, moisture content; DM, dry matter content; TA, titratable acidity. All the values are means of three replications +SD.

Moisture content decreased from 85.24% for fresh apricot to 21.05% and 26.06% for TSD and OD dried apricots, respectively. As a result, dry matter and ash content decreased after the drying processes, as well as TA. However, a slight increase in pH was observed for the dried apricots (Table 1).

Total Carotenoids (TC) were assessed before and after the drying processes. Data are reported on a DM basis in figure 2. Significant differences between the TC of fresh and dried apricots ($p<0.0001$) were reported. OD had remarkably affected TC content of apricots; the drying process decreased TC from 46.4 ± 6.2 mg β-CE/100 g DM to 25.8 ± 2.4 mg β-CE/100 g DM, which represents a loss of 44%. Furthermore, compared to OD, TSD was much harmful to TC, where the traditional process provoked a more significant decrease of 67%.

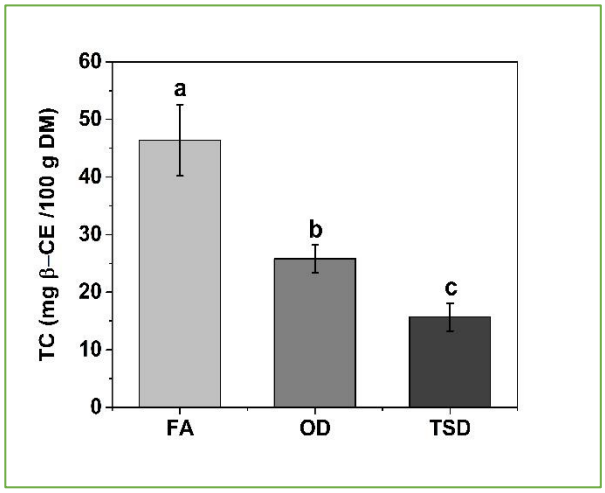

Figure 2: Effects of oven-drying (OD) and traditional sun-drying (TSD) on apricot total carotenoids (TC)
FA, fresh apricot; β-CE, β-carotene equivalent; DM, dry matter content. All the values are means of three replications +SD. The same letters indicate the absence of significant differences ($p<0.05$).

The effects of TSD and OD on total phenolic compounds (TPC), total flavonoids (TF), total tannins (TT), and Free radical scavenging activity (FRSA) of apricots are reported on a DM basis on figure 3. All measured parameters were significantly affected ($p<0.05$) by both drying methods (TSD and OD). TSD decreased remarkably the amount of TPC (Figure 3.a), TF (Figure 3.b), and TT (Figure 3.c) of apricots. The drying process caused significant losses of 15%, 43%, and 36%, respectively. However, for the OD, a decrease of 12% in TT after the drying process was recorded, while a slight increase of 4% was observed in TPC and TF.

FRSA (%) results are presented on figure 3.d. In contrast to our previous results (TPC, TF, and TT), FRSA increased significantly in dried apricot, and the heist FRSA was reported for the TSD. The methanolic extract of the dried apricot showed a radical scavenging activity of 40.1 ± 0.8%, followed by OD (35.9±1.7%), and fresh apricot (31.7 ± 1.2%).

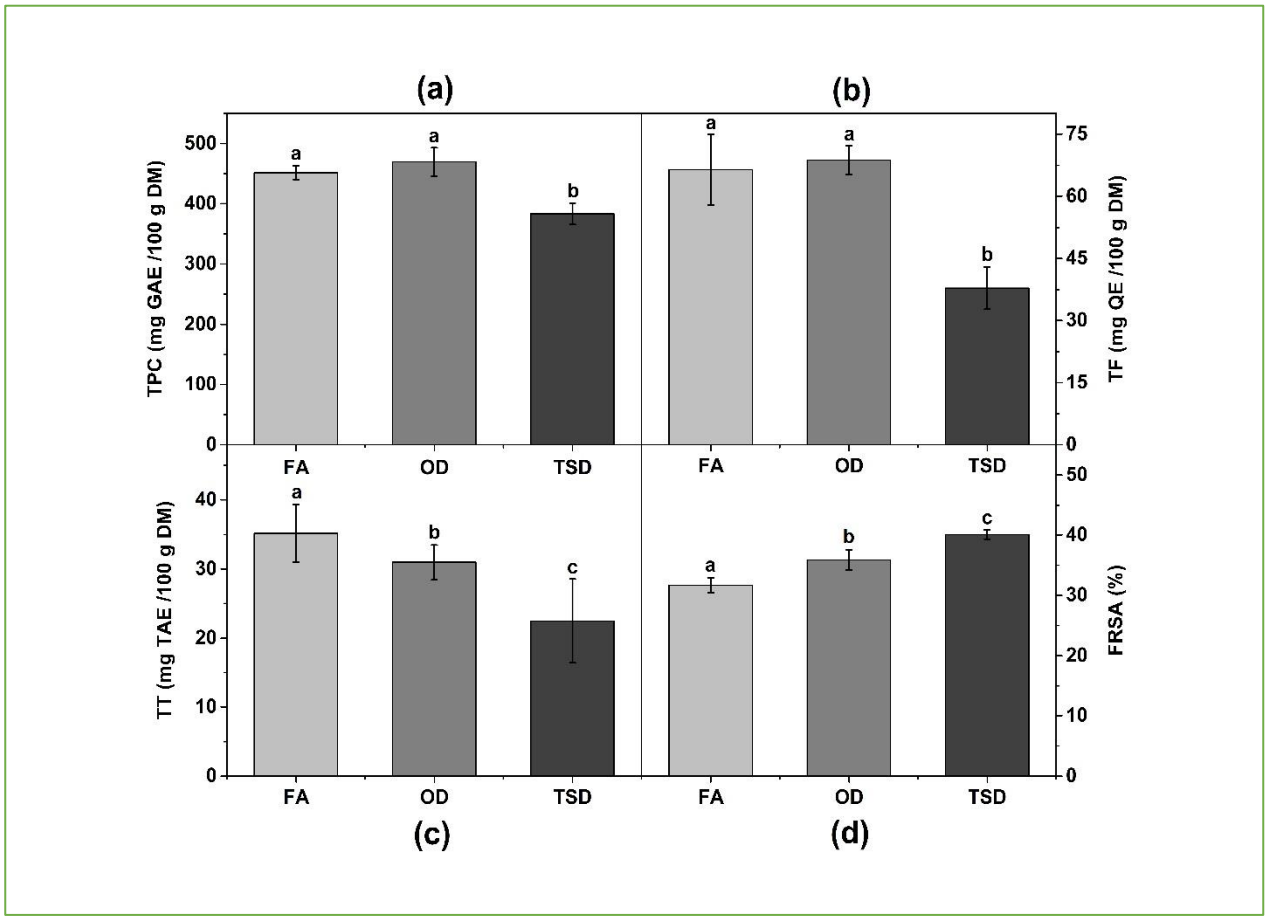

Figure 3: Effects of oven-drying (OD) and traditional sun-drying (TSD) on apricot total phenolic compounds (TPC), total flavonoids (TF), total tannins (TT), and Free radical scavenging activity (FRSA)

a: TPC, b: TF, c: TT, d: FRSA. FA, fresh apricot, DM, dry matter content, β-CE: β-carotene equivalents, GAE: gallic acid equivalent, QE: quercetin equivalent, TAE: tannic acid equivalent. All the values are means of three replications +SD. The same letters indicate the absence of significant differences ($p<0.05$).

4. DISCUSSION

The comparison between our findings and the different works on apricots showed that our values are included in the ranges reported by Akin *et al.* [26] for the humidity (74.19 - 88.17%) and ash content (0.50% - 0.89%), and the intervals indicated by Leccese *et al.* [6] for pH (3.35 - 4.41) and acidity (0.48 - 2.28%). The important loss of water during the drying process resulted in a concentration of the different components of apricot, which explains the increase in ash content and acidity (Table 1). The titratable acidity of apricot after the TSD (3.62%) was higher than the titratable acidity of apricot after the OD (2.87%). This can be due to the difference in drying conditions (mainly time and temperature) between the two processes, where apricot (climacteric fruit) can carry on the post-harvest maturation during TDS [11]. The drying temperatures (40°C) during TDS promote the physiological and biochemical reactions, including organic acids synthesis. However, the drying temperatures (65°C) during OD are relatively high, thus the physiological and the biochemical reactions are restrained. High acidity for sun-dried apricots was also reported by Madrau *et al.* [15] (4.67 - 7.33%) and Bolin [27] (3.8%). The ash content of the traditional sun-dried apricots was superior then the ash content of the oven-dried apricots too. This difference is mostly caused by the salt added during the process. Contamination by dust during the TSD may have also contributed to the ash content increase. Both drying methods have significantly decreased the carotenoids content of apricots (Figure 2). OD decreased apricot carotenoids by 44% of the initial content. These results are comparable to those of Fratianni *et al.*[16], where they reported significant losses of 50 % in total carotenoids for apricots dried at temperatures between 60 and 70 °C. In the same context, Karabulut *et al.* [17] recorded a decrease of 40% in β-carotene content after drying at 70 °C, and surprisingly they reported more losses (60%) by decreasing the drying temperature to 60 °C. The thermal damages caused by drying were directly proportional to the temperature used and the time operated in the process. It was mentioned that the heat applied during drying softens the cell walls, making them fragile and easily separated [16,17]. Therefore, the

carotenoids, usually stable within the original structure, become highly sensitive to external agents such as heat, oxygen, and light [16]. However, carotenoids were more sensitive to TSD. The traditional process caused the most significant losses, where the measurement of total carotenoid has highlighted a more significant decrease of 67% compared to OD. It has been reported that oven drying and additional conventional drying methods have several advantages over sun-drying. García-Martínez *et al.* [19] related these advantages to the fact that conventional drying methods are more rapid and the fruits are not in contact with the open environment during the process. Our results are consistent with those of Korekar *et al.* [28], where the authors reported a loss of 65% of β-carotene content for the sun-dried apricots. Furthermore, in a recent study, performed by Vega-Gálvez *et al.* [18] on the effect of hot-air drying temperatures (40-80°C) on apricot bioactive compounds, the results showed that the increase of drying time led to more damage than the increase of temperature. Moreover, the same authors reported the more significant carotenoids content decrease (53% loss) at the lowest temperature (40°C). The principal cause of carotenoids degradation, during the TSD, was the direct exposure of apricot to oxygen and sunlight. The destructive effect of oxygen and sunlight on carotenoids was confirmed by several authors [16-19, 29]. The exposure to oxygen during drying causes the generation of peroxides and oxidizing free radicals, which can cause a serious carotenoids loss [29]. Yang *et al.* [30] reported that carotenoids are sensitive to oxidation and can decompose, even if the samples were kept in the presence of traces of oxygen. The degradation of carotenoids can also result from photo-oxidation in the presence of light [31].

The investigated apricots in the current study were found to be a suitable source of phenolic compounds (451.6 ± 11.3 mg GAE / 100 g DM). The comparison with other studies shows that our results are higher than the ranges (319 and 413 mg GAE / 100 g DM) as reported by Milošević *et al.* [32]. As shown on figure 3.a, drying methods affected significantly apricots' phenolic compounds. In the last decades, numerous works investigated the effect of different treatments on phenolic compounds. Yet, data are not to seem correlated and even contradictory [33]. Most researchers reported a negative effect (decrease in phenolic compounds concentration) of heat treatments on phenolic compounds [13, 34,35]. On the other hand, several authors stated an increase in phenolic compounds after heat treatments. In our study, we witnessed both effects, where OD caused a slight increase in TP and TF, while TSD led to a significant decrease in TP, TF, and TT.

The increase, detected in TPC and TF after OD, was also reported in a previous study on apricot by Hussain *et al.*[36], where the authors detected an increase of 11.6-16.4% in the phenolic compounds concentration after drying. In the same angle, Santos *et al.* [37] studied the effect of drying at 60 °C on the phenolic compounds of pears, and reported an increase of 2.4-15% in TPC. However, Madrau *et al.* [15] reported a decrease in TPC of apricots dried at a lower temperature (55°C). In addition, Vega-Gálvez *et al.* [18] reported a significant decrease of TPC (>73%), and TF (>61%) for dried apricots at temperatures between 60 and 70 °C. The increase of TPC and TF can be explained by the improvement of phenolic compounds extractability, due to the relatively high temperature (70 °C) during the drying process, where it has been reported that high temperature facilitates the extraction of phenolic compounds [38-40]. Phenolic cosmpounds occur more often conjugated in soluble and insoluble forms, covalently bound to structural components of the cell wall (cellulose, hemicelluloses, and lignin). Bound phenolic compounds constitute an average of 24% of the fruits TPC, and heat treatments are likely to release those bound phenolic compounds [41]. Brunton [42] reported that, in addition to the better extractability of phenolic compounds during drying, the increase of TPC and TF can be due to the depolymerization of phenolic compounds with high molecular weights such as tannins. This statement agrees with our findings, where the increase of TPC and TF after OD was accompanied by a decreased in TT.

Unlike OD, TSD has caused significant losses of apricots phenolic compounds, where TPC, TF, and TT decreased by 15%, 43%, and 36%, respectively. This decrease can be explained by the enzymatic oxidation of phenolic compounds. Madrau *et al.* [15] reported that drying apricots for a long period in the presence of oxygen promotes the degradation of phenolic compounds by polyphenol oxidase (PPO). This enzyme is responsible for the oxidation of phenolic compounds to quinines. The enzymatic oxidation is followed by non-enzymatic polymerization of the resulted quinones into dark/brown polymers called melanins [43]. Apricot PPO remains active at drying temperature below 55 °C [44], and since TSD was performed at temperatures ~ 40 ° C, enzymatic browning is mostly the main cause of phenolic compounds decrease in TSD apricots. This was also visually confirmed, where the dried apricots had dark colors. Our results are in agreement with the results obtained by Vega-Gálvez *et al.* [18], where the authors attributed the decrease in TPC and TF observed during drying at 40 °C to the PPO activity.

The antioxidant activity increased significantly after the OD, this can be explained by the increase reported previously in TPC and TF, where phenolic compounds are known for their FRSA. However, while the expectations shifted toward a decrease in FRSA after TSD, surprisingly, the results showed a significant increase in FRSA (Figure 3.d). These results are similar to those of Madrau *et al.* [15] and Hussain *et al.* [36], the authors reported a significant increase in FRSA for dried apricots, despite the reduction in TPC. However, Vega-Gálvez *et al.* [18] reported a decrease in FRSA for dried apricots at temperatures between 40 and 80°C. According to Pokorny & Schmidt [45], FRSA of processed fruits may be enhanced by the development of new antioxidants, such as the products of browning reactions. Furthermore, Gan *et al.* [46] reported an increase in the antioxidant activity for the dried mung bean, which was also accompanied by an increase in browning. The same findings were reported by Lee *et al.* [47] for dried onions, the authors indicated that the antioxidant activity depends more on browning during drying than on phenolic content.

5. CONCLUSION

The main obtained results showed that TSD conditions, such as direct sunlight exposure and the longtime of the drying process, are parameters that affect negatively carotenoids and phenolic compounds of apricots. These conditions promoted the carotenoids photo-oxidation and the enzymatic oxidation of phenolic compounds (by PPO). Thus, OD caused less damage to carotenoids and phenolic compounds compared to TSD. The advantages of OD are the short period of drying, and the fruits are not exposed to open air, which has resulted in better preservation of carotenoids and phenolic compounds. However, despite the destructive effect of TSD on carotenoids and phenolic compounds, the process increased the FRSA of the phenolic extract. The enzymatic oxidation of the phenolic compounds during TSD promoted the generation of new compounds with high antioxidant properties. Thereby, the traditionally sun-dried apricots are a better source of antioxidants.

Acknowledgment

The authors wish to thank the Algerian Ministry of Higher Education and Scientific Research (MESRS) for the financial support of this work.

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Cite this article as: Derardja A and Barkat M. Effect of traditional sun-drying and oven-drying on carotenoids and phenolic compounds of apricot (*Prunus armeniaca* L.). *Nor. Afr. J. Food Nutr. Res.* July - December (2019);03(06):186-94. https://doi.org/10.5281/zenodo.3497409

The North African Journal of Food and Nutrition Research: (2019)03;(06):195-200
https://doi.org/10.5281/zenodo.3540383

Original Article

OPEN ACCESS

Making Nutrition a Development Priority in Africa

eISSN: 2588-1582

Contents lists available at

Journal homepage: https://www.najfnr.org

Beneficial Effects of a Fermented Maize product with Its Supernatant, *Lactobacillus fermentum* and *Lactobacillus brevis* in Rat Model of Colitis

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ARTICLE INFO

Article history:
Received 10 July 2019
Accepted 10 November 2019
Published 14 November 2019

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Access this article online

Quick Response Code

https://doi.org/10.5281/zenodo.3540383

Article edited by:
Pr. A. Mobolaji OMEMU
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ABSTRACT

Background: African fermented foods such as maize cereal (*Ogi*) and its supernatant (*Omidun*) are reservoirs of beneficial lactobacilli and carboxylic acids. Based on their constituents, their beneficial effect in a rat model of 2,4,6-trinitrobenzene sulphonic (TNBS) acid-induced colitis was assessed in comparison with two lactobacilli in a rat model of colitis. **Methods**: Female Wistar rats were distributed into seven groups of 5 rats each; the rats were pretreated for 14 days prior to colitis induction with *Ogi*, *Omidun*, *L. fermentum*, *L. brevis* and 7 days post colitic induction. Colitis was induced by an intracolonic administration of TNBS. The response of the rats to treatment was assessed macroscopically and biochemically. **Results**: Treatment with the Dexamethasone, *Ogi* and *Omidun* resulted in a significant reduction in colonic damage score and weight/length ratio ($p<0.05$). Treatment with *Ogi*, *Omidun*, *L. brevis*, and dexamethasone significantly prevented depletion of colonic glutathione and superoxide dismutase. The up-regulation of myeloperoxidase activity was inhibited in all treated colitic rats ($p<0.05$). However, *Ogi* appears to produce a better protective effect than the other treatment groups. **Conclusions**: This study reports that *Ogi* protects Wistar rats against the deleterious effect of trinitrobenzene sulphonic acid better than pure lactobacilli strains.

Keywords: fermented food, lactic acid bacteria, inflammation, oxidative stress.

1. INTRODUCTION

Inflammatory Bowel Disease (IBD) is a chronic, idiopathic inflammatory disease of the gastrointestinal tract [1]. In healthy individuals, immunological tolerance to the gut microbiome is maintained, whereas in those with IBD, these homeostatic mechanisms remain disrupted [2]. Furthermore, the composition of the intestinal flora in patients with IBD is altered when compared with healthy individuals, resulting in a general loss of diversity [2]. It has been shown that dysbiosis can lead to a qualitative and quantitative decrease in the mucosal barrier and consequently, translocation of pro-inflammatory substances into the colon, which could be responsible for inflammation and subsequently colitis [3]. Therefore, there is currently a strong interest in exploring beneficial bacteria in the treatment of colitis.

Lactic acid bacteria (LAB) are found in food and contribute to the healthy microbiota of human intestinal mucosal surfaces. Traditional fermented products are one of the primary sources of LAB [4-6]. In developing countries where probiotic products are not readily available, the next best option is fermented foods. Fermented cereal is consumed worldwide and provided different names in different countries. In Nigeria, *Ogi* is the name attributed to fermented cereal e.g. maize, sorghum, etc. This fermented cereal is frequently consumed as a meal for breakfast by many tribes. Occasionally, Raw *Ogi* (the slurry) is

administered as a local fermentation product remedy against diarrhea, especially in rural areas with little access to proper health care [7]. *Omidun*, the supernatant obtained from the raw *Ogi* slurry, is locally utilized as a solvent to soak the bark or root of some plants and for treating fever and malaria [8]. *Ogi* has been shown to contain eleven carboxylic acids with butyric acid, lactic acid, and acetic acid as the major constituents [9]. In addition, *Ogi* and *Omidun* contain several species of beneficial LAB. We have previously reported isolation and identification of *Weissella paramesenteroides*, *L. brevis*, *L. rossiae*, *L. fermentum, L. plantarum*, *Acetobacter pasteurianus* and *Paenibacillus sp.* in different varieties of *Ogi* and *Omidun* with interesting antibacterial properties [10].

To the best of our knowledge, no published data on beneficial effects of *Ogi* and *Omidun* in comparison to lactobacilli strains on colitis are available. Thus, this study assessed the beneficial effect of *Ogi*, *Omidun* and two pure lactobacilli strain in a rat model of colitis.

2. METHODS

2.1. Bacterial Strains

Lactobacillus brevis FA021 and *Lactobacillus fermentum* FA020 has been previously isolated and selected because of their substantial antibacterial properties. Those target bacteria were used in this study because the source of isolation was human and displaying antibacterial properties. For this *in vivo* rat study, they were subcultured on de Man, Rogosa Sharpe broth (Oxoid, UK) and incubated at 37ºC for 24-48hrs under microaerophilic conditions after which they were centrifuged at 4000 rpm for 6 minutes. The supernatant was decanted off and the bacterial pellets were washed with normal saline and re-suspended in 0.5 ml of normal saline.

2.2. Collection of Ogi Slurry and its Supernatant (*Omidun*)

Maize used for the production of *Ogi* slurry and *Omidun* was purchased from a local vendor at Agbowo, Ibadan, Oyo State, Nigeria. The vendor made *Ogi* by soaking maize grains in water for 48 hours and then wet milling before fermentation at room temperature for 24 hours; thereafter, appropriate volumes of the supernatant (*Omidun*) and *Ogi* were collected and used for the analysis. Samples were handled within 72 h after which another fresh batch was collected to maintain the 72-h interval use. The colony-forming units/ml for every fresh sample was analyzed by plating out 1 ml of Omidun and 1 g *Ogi* in MRS agar (Oxoid, UK) after appropriate dilutions were made in saline solution.

2.3. Ethical Consideration

Experimental procedures and protocols used in the current study conform to the "Guide to the care and use of animals in research and teaching" (NIH publications number 85-93 revised in 1985).

2.4. Rat Weight Analysis

The body weight of experimental animals was measured weekly. After two weeks of experimental feeding, colitis was induced and the final weight measured at the third week.

2.5. Experimental Animals

Thirty-five healthy female Wistar albino rats were obtained at 4 weeks from the Animal House, Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan. The rats were housed in Makrolon cages in a well-ventilated animal house facility at room temperature (28 + 2ºC). They were fed with standard rat pellets (Ace Feeds Nigeria Limited) and water *ad libtum* until they reached the target weight for the study (160-180 g). Experimental rats were randomly distributed into seven groups of five rats each. Four of the seven groups were pre-treated with *L. brevis* (0.5ml of 1.21 x 10^8 CFU/ml), *L. fermentum* (0.5ml of 3.8 x 10^7 CFU/ml), *Ogi* (1.25 mg/ml of 8.6 x 10^8 CFU/ml) and *Omidun* (0.5ml of 1.25 x 10^7cfu/ml) for 13 days (D0-D12), before induction of colitis on day 13 (D13) and for seven days post colitic induction. The remaining three groups served as controls: dexamethasone-treated colitic rats (4mg/ml), untreated colitic rats and healthy non-colitic rats.

2.6. Induction of Colitis

Colitis was induced in 30 out of 35 rats using a previously reported method [11]. Momentarily, food was withdrawn 18h prior to the induction of colitis. Rats were anesthetized with ketamine (50 mg/kg) and diazepam (2.5 mg/kg). Colitis was induced by a single intracolonic administration of 0.25 mL of 40 mg/mL trinitrobenzenesulfonic acid dissolved in 50% ethanol) into the distal colon by means of a soft pediatric catheter introduced 8 cm into the anus. The animals were maintained in a head-down position for 5 minutes to recover from anesthesia and return back to their cages.

2.7. Assessment of Colonic Damage and Response to Treatment

Rats were euthanized by an overdose of ether anesthesia at the seventh day post-colitic induction. The distal colon was excised, luminal contents flushed with cold normal saline and colon placed on ice. Thereafter, each colon was opened by an incision along the mesenteric border, the body weight and length measured, and the disease severity scored. Disease severity was assessed using a standard scoring system [12], scored on a scale of 0 – 10 depending on the severity of the inflammation and ulcer.

Table 1: The weight of Wistar rats before and after the induction of colitis and treatment Weight

WEEK	Weight (g)						
	L. fermentum	*L. brevis*	*Omidun*	*Ogi*	Dexa.	Neg.control	Non-colitic
1	172 ± 3.0	174.4±3.3	170.4±2.9	164.1±8.6	164.3±5.3	159.4±6.7	161±2.9
2	178.6 ±7.5	178.6±7.4	178.8±4.1	168.4±7.3	169 ± 5.1	169.9±6.6	165.8±3.0
3	173.4±9.43	163.6±6.5	166.9±5.1	159.9±1.6	162.9±4.0	157.2±3.9	175.4±4.81
% W.L	2.9*#	8.4#	6.6#	4.8*#	4.1*#	7.5**	0

Dexa – dexamenthasone, Neg.control - untreated colitic rats, * p<0.05 (treatment groups vs. TNBS control group). # p<0.05 (Healthy group vs. treatment groups), ** TNBS control group vs healthy group, WL.: weight loss.

Thereafter, for biochemical assays, samples were cut from the entire colon.

2.8. Sample Preparation for Biochemical Assays

A known weight of freshly excised tissue was homogenized in HTAB buffer (50 mg/ml) and centrifuged at 12,000 rpm for 10 minutes at 4ºC. Two hundred microliter of the supernatant was collected and the remaining homogenized samples was stored at -20ºC. The supernatant was used to estimate the total glutathione content (GSH), and Superoxide Dismutase (SOD). GSH level was estimated according to the method previously described [13] and the results were expressed as nanomoles per gram of wet tissue. A method described by Mistra & Fridovich [14] was used to evaluate the superoxide dismutase (SOD) content of the colon samples. The remaining frozen homogenized colon sample was allowed to undergo two cycles of freezing and thawing. Thereafter myeloperoxidase activity (MPO) was quantified by a method previously described [15]. The results were expressed as MPO units per gram of wet tissue; one unit of MPO activity was defined as that degrading 1 mmol hydrogen peroxide/min at 25°C.

2.9. Statistical analysis

Data obtained were expressed as the mean ± SEM. Differences between means were tested for statistical significance using a one-way analysis of variance (ANOVA) with the Tukey post hoc test. P-value <0.05 was considered significant.

3. RESULTS

There was an increase in the body weight of the rats, during the first two weeks of the experiment prior to colitis induction (Table 1). All colitis induced rats lost weight immediately after induction, gaining weight gradually. At the end of the third week, the colitic rats treated with the *L. brevis* group had the highest weight loss (8.9%) while those treated with *L. fermentum* group had the least weight loss (2.9 %). Colonic damage in experimental animals evidenced by the size of lesions, number of ulcers as well as hyperemia, were scored on a scale of 0-10 as presented in Table 2. Treatment with the standard drug (dexamethasone), *Ogi* and *Omidun* respectively resulted in a significant reduction in colonic damage score (4.0 ± 0.71, 4.15 ± 0.29 and 4.4 ± 0.31) in comparison with untreated colitic group (6.0 ± 0.41, p≤0.04). Similarly, the colonic weight/length ratio increased significantly in untreated colitic rats (170.7 ± 6.9 mg/cm) in comparison with non colitic rats (113.3 ± 2.8 mg/cm, p<0.0001). Treatment with Dexamethasone, *Ogi* and *Omidun* resulted in a significant reduction in colonic weight/length ratio (105.5 ± 6.9, 117.15 ± 9.1 and 129.3 ± 10.54 mg/cm) in comparison with untreated colitic group (170.7 ± 6.9 mg/cm p≤0.011). Furthermore, a significant reduction of myeloperoxidase activity (MPO) was observed in all treated colitic rats (3.05 ± 0.15 - 3.8 ± 0.08 U/mg tissue) with *Ogi* producing the best effect when compared with untreated colitic rat (4.51 ± 0.10 U/mg tissue, p≤0.007 (cf. Table 3). In addition, about 4-fold depletion of colonic glutathione level was observed in untreated colitic rats (56.18±5.49 nmol/mg tissue) in comparison with non-colitic rats (202.3 ± 3.97 nmol/mg tissue, p<0.0001 (cf. Table 3). However, treatment with, *Ogi*, *L. brevis*, *Omidun,* and dexamethasone significantly prevented depletion of colonic glutathione in colitic rats in comparison to untreated colitic rats (119.4 ± 2.65 vs 203.6 ± 16.83 nmol/mg tissue p≤0.0001). Interestingly, the GSH level in colitic rats treated with *Ogi* was identical to the non-colitic healthy rats (p=0.94; Table 3).

Table 2: Effects of treatment on macroscopic colon damage score and colonic weight/ length ratio in colitic rats

Treatment Group	damage score (0–10)	weight/length (mg/cm)
Healthy (non-colitic)	0.0 ± 0.0	113.3 ± 2.8
Colitic control	6.0 ± 0.41**	170.7 ± 6.90**
Dexamethasone	4.0 ± 0.71#	105.5± 6.90*
L. fermentum	5.25 ± 0.25#	175.3 ± 5.70#
L. brevis	5.00 ± 0.41	150.8 ± 15.57#
Omidun	4.43 ± 0.31*#	129.3 ± 10.54*
Ogi	4.15 ± 0.29*#	117.1 ± 9.10*

Macroscopic damage (Mean ± SEM). *p<0.05 (treatment groups vs. TNBS control group), #p<0.05 (healthy group vs. treatment groups), ** TNBS control group vs healthy group.

Similarly, a significant 6-fold depletion of colonic SOD activity was observed in untreated colitic rats (0.013 ± 0.001 U/g tissue) in comparison with non colitic rats (0.08 ± 0.002 U/mg tissue, p<0.0001). In dexamethasone, *Omidun* and *Ogi* treated groups, a significant reduction in the depletion of SOD was observed (0.031 ± 0.002 vs 0.035 ± 0.004 U/g tissue p≤0.0007) with *Ogi* showing the highest reduction in the depletion of SOD.

Table 3: Biochemical Analysis of Rats Samples

Treatment Group	MPO (U/mg tissue)	GSH (nmol/mg tissue)	SOD (nmol/mg tissue)
Healthy (non-colitic)	0.98±0.07	202.3±3.97	0.079 ± 0.002
Colitic control	4.51±0.10**	56.18±5.49**	0.013 ± 0.001**
Dexamethasone	3.76±0.07	131.1±7.24*#	0.031 ± 0.002*#
L. fermentum	3.35±0.31*#	66.21±3.3#	0.022 ± 0.009#
L. brevis	3.46±0.12*#	119.4±2.65*#	0.012 ± 0.001#
Omidun	3.8±0.075*#	152.2±11.76*#	0.031±0.003*#
Ogi	3.05±0.15*#	203.6±16.83*	0.035± 0.0024*#

Data are expressed as Mean ± SEM. * p<0.05 (treatment groups vs. TNBS control group), # p<0.05 (Healthy group vs. treatment groups), ** TNBS control group vs healthy group.

Assessment of difference between *Omidun*/*Ogi* in comparison to *L. brevis* and *L. fermentum* in MPO, GHS, SOD, damage score, weight length ratio revealed that activities of *Ogi* or *Omidun* is significantly better in all tested parameter (GSH, p=0.0001, 0.03. SOD, p=0.005, 0.0003, damage score p=0.02, Weight/length ratio, p=0.005, 0.0006) except MPO where *L. brevis* was better than *Omidun* (p=0.04).

4. DISCUSSION

In the present study, dexamethasone, *Ogi* and *Omidun* were able to ameliorate the macroscopic colonic damage caused by intracolonic delivery of TNBS in colitic rats. In contrast, treatment with *L. brevis* and *L. fermentum* showed non-significant protection against macroscopic damage to the colon. An increase in colon weight/length ratio is an indication of intestinal edema, a symptom of inflammation [16]. Significant reduction in colonic weight/length ratio as observed in colitic rats treated with dexamethasone, *Ogi* and *Omidun*, signified resolution of intestinal edema, a symptom of inflammation. Furthermore, the up-regulation of MPO, an indicator of neutrophil infiltration, was moderately inhibited in colon tissues in the treatment groups. Prevention of access of neutrophils to the inflamed areas of colon could prevent the release of proteases [17]. The inhibition of the up-regulation of myeloperoxidase enzyme could also be an indication of successful gut microbiota repopulation by *Ogi*, *Omidun*, *L. brevis,* and *L. fermentum*. It has also been shown that proteases produced by members of the gut microbiota could prevent the activation of an immune response and consequently inflammation by degrading the antigenic structure of most antigens [18]. However, dysbiosis of the gut microbiota eliminates much of this function, allowing the successful penetration of antigens and antigenic products and subsequently inflammation.

Ogi and its constituents are reportedly dominated by LAB with the identification of *L. fermentum*, *L. plantarum*, *L. pantheris,* and *L. vaccinostercus* [10,19]. Consumption of *Ogi* might be beneficial because of the ability of LAB to modulate intestinal microbiota. The better activities of *Ogi* and *Omidun*, in comparison to single cultures of LAB used in our study, may be due to the synergistic effects of different lactobacilli species in *Ogi* and *Omidun* [6,10]. Moreover, different beneficial bacteria have been reported to decrease the intestinal microbiota imbalance induced by IBD when used simultaneously. VSL#3, a probiotic mixture of *Streptococcus thermophiles*, *L. plantarum*, *Bifidobacterium brevis*, *B. longum*, *B. infantis*, *Lactobacillus acidophilus*, *L. paracaseiand*, *L. bulgaricus*, and has been successfully used as adjunctive treatment to treat ulcerative colitis [20].

Our findings suggest that *Ogi*, *Omidun*, *L. brevis* and to a lesser extent *L. fermentum* prevent the depletion of GSH and might be useful to improve antioxidant status in colitis. It is known that some lactobacilli are able to produce antioxidants that help eliminate oxidative stress produced by reactive oxygen species [21]. *Lactobacillus fermentum* has also been previously shown to contain a notable level of reduced glutathione [22]. The increased colonic superoxide dismutase activity observed, is indicative that the individual lactic acid bacteria, as well as their presence in combination in Ogi and Omidun, reduced oxidative stress thereby preventing exacerbation of inflammation. A previous report has demonstrated the ability of *Lactobacillus lactis* and *Lactobacillus plantarum* to produce superoxide dismutase enzyme and ameliorate experimental colitis [23].

The anti-inflammatory and antioxidant activities shown by *Ogi*, *Omidun*, and to a lesser extent by *L. brevis* and *L. fermentum* may be due to their ability to prevent the adhesion of harmful bacteria to the intestinal epithelium by competing for nutrients. Members of the genus *Lactobacillus* have been shown to be able to adhere to intestinal mucus and extracellular matrix through various adherence factors, such as those with mucin binding domains [24]. An altered mucus layer, which is a prominent feature in colitis, could interfere with the binding ability of LAB and consequently be responsible for the dysbiosis of the intestine LAB population.

5. CONCLUSION

This study suggests that *Ogi*, *Omidun* and to a lesser extent, *L. fermentum* and *L. brevis* produce beneficial effects against TNBS induced colitis. The activities of fermented food products are better than single lactobacilli cultures. The limitations of the study are not ascertaining the mechanism of action of Omidun against the colitic rats and also, the study investigated only two LAB strains

Funding

This research was funded by the International Capacity Building Fund from Society for Applied Microbiology (SFAM) obtained by Funmilola A. Ayeni in 2015.

Acknowledgment

The technical assistance of Mrs. Sakirat Braimah and Ms. Adedunke Oshinloye is highly appreciated.

Conflict of Interests

The authors declare no conflict of interest.

6. REFERENCES

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Cite this article as: Audu H.J, Abiodun O.A and Ayeni F.A. Beneficial Effects of a Fermented Maize product with Its Supernatant, *Lactobacillus fermentum* and *Lactobacillus brevis* in Rat Model of Colitis. *Nor. Afr. J. Food Nutr. Res.* July - December (2019);03(06):195-200. https://doi.org/10.5281/zenodo.3540383

The North African Journal of Food and Nutrition Research: (2019)03;(06):201-05
https://doi.org/10.5281/zenodo.3542124

Short Communication

OPEN ACCESS

Making Nutrition a Development Priority in Africa

eISSN: 2588-1582

Contents lists available at

Journal homepage: https://www.najfnr.org

Quantitative Analysis of Proanthocyanidins (Tannins) From Cardinal Grape (*Vitis vinifera*) Skin and Seed by RP-HPLC

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ARTICLE INFO

Article history:
Received 14 September 2019
Accepted 10 November 2019
Published 16 November 2019

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Access this article online

Quick Response Code

https://doi.org/10.5281/zenodo.3542124

Article edited by:
Dr. Lynda ARKOUB-DJERMOUNE
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ABSTRACT

BACKGROUND: Grape phenolics are structurally diverse, from simple molecules to oligomers and polymers usually designated "tannins or proanthocyanidins (PAs)" referring to their ability to interact with proteins. Those compounds have been attributed to a great number of biological activities beneficial for human health as they act as antioxidant, anti-inflammatory, antitumor, etc. **AIMS**: The objective of the current study was to quantify and to identify the PAs and determine the mean degree of polymerization (DPm) in seeds and skins of the grape cardinal variety cultivated in El-Tarf region, Algeria. **METHODS AND MATERIAL**: To determine PAs, Reverse Phase High-Performance Liquid Chromatography with Diode Array Detection *(RP-HPLC-DAD)* has been utilized. The DPm was determined after the reaction of thiolysis in the presence of toluene-α-thiol reagent. **RESULTS**: HPLC-DAD analysis of Cardinal skin and seed extract showed that epicatechin gallate (ECG) and epigallocatechin (EGC) were the major constitutive units of grape skin tannins and the mean degree of polymerization (DPm) was lower for seed PAs than for skin. **CONCLUSIONS**: This study showed the richness of skin and grape seeds in polyphenolic compounds (PAs). Therefore, these parts of grape can be used as a potential source of bioactive molecules to promote the health of populations in this region in Algeria.

KEYWORDS: Grape, Skin, Seed, Proanthocyanidins, RP-HPLC-DAD.

1. INTRODUCTION

Condensed tannins, also called proanthocyanidins (PAs), constitute the most abundant class of phenolics in grape berries [1]. Those compounds represent a class of phenolics that take the form of oligomers or polymers of polyhydroxy flavan-3-ol units, such as (+)-catechin and (-)-epicatechin. The grape (*Vitis vinifera*) especially seeds are a rich source of PAs. These grape seed PAs are mainly dimers, trimers, and highly polymerized oligomers of monomeric catechins. PAs act as powerful antioxidants with beneficial effects for human health including protection against free radical-mediated injury and cardiovascular disease and exhibit a strong antitumor and antimicrobial activity [2-4]. Furthermore, PAs contribute to the astringency and taste of many fruits and other plant products, such as fruit juices, tea and wine through interactions with salivary proteins [5]. The oligomeric and polymeric PAs may contribute significantly to grape tannin composition. To the best of our knowledge, no study involving PAs quantification and qualification has been published on grape grown in the region of North-East Algeria. Thus, the objective of the current study was to quantify and identify the grape skin and seed PAs from Cardinal grape, that is a purple-colored table grape cultivar, using RP-HPLC-DAD-UV-VIS method, and the DPm after thiolysis reaction using the toluene-α-thiol reagent. The PAs compositions of grape extracts (seed and skin) and the DPm from Cardinal cultivar were then compared and discussed.

2. MATERIAL AND METHODS

2.1. Grape sample

Approximately, (2 kg) of grape Cardinal (red variety) was collected in late summer 2012 in the region of El-Tarf located in North-East of Algeria (36° 45' 00" N; 81° 10' 00" E). The experimental vineyard was raised in 1980. The distance of sowing was 3 ×1 m, with two rows support, and the training system was a "double-branched asymmetrical cordone". The sample was collected at commercial maturity with the Brix values of 17.65 °Brix. The sampling of grapes was done meticulously and berries were collected randomly from top, bottom, sun-exposed and unexposed clusters on each side of the vine. The sample was placed in clean, dry, plastic boxes and quickly transported and stored until analysis.

2.2. Sample treatment

Before extraction, skin and seed were manually separated from the whole grape berries and dried at oven temperature of 50°C until constant weight. The dried skin and seed were then crushed in a domestic mill for 2 min and then used for extractions.

2.3. Extraction and isolation of skin and seed PAs

According to Brossaud *et al.* [6], the extraction procedure was as follow: dried skin powder (2 g) was successively extracted twice with 80 mL of methyl alcohol/ water /TFA (80:20:0.05) and afterward twice with 50 mL of a mixture acetone/water (60:40) (25°C/15 min/ 250 rpm). Dried seed powder (0.1 g) was then extracted by maceration in 50 mL of a mixture of acetone/water (60:40) and 300 µL of methyl-4-hydrobenzoate (1g/L), with stirring for 70 min. Both extracts were centrifuged (10°C/10 min/10,000 rpm) and the supernatants were then filtered through glass microfiber filter GF / A 1.6 µm before drying under vacuum at 30°C and dissolved in 5 mL of methanol to yield a crude skin and seed PAs extracts, respectively. The extracts were chromatographed on Fractogel Toyopearl® HW-40(F) (300 mm × 10 mm i.d.) (Tosoh Corporation, Japan) to eliminate anthocyanins, flavonols, monomeric and dimeric flavanols with 30 mL of ethyl alcohol/water/TFA (11:9:0.001). The tannin fraction was eluted with acetone/water (6:4) (30 mL). A quantity of 300 µL of internal standard (50 mg of methyl 4-hydroxybenzoate in 100 mL of methanol) was added. The acetonic fraction was dried using a rotary evaporator Bucchi® under vacuum at 30°C and then dissolved in 5 mL methanol for the thiolysis reaction.

2.4. Characterization of polymeric PAs

The characterization of condensed tannins by depolymerization is frequently employed. 120 µL of each fraction was placed in a glass ampoule with an equal volume of reagent toluene-α-thiol. After sealing, the mixture was shaken and heated at 90°C for 2 min, then quickly cooled with cold water, to stop the reaction. This technique allows the distinction between the terminal units released in the form of flavan-3-ols, the intermediate and upper units released in the form of benzyl thioethers [6]. The thiolysis reaction medium (20 µL) filtrated through a membrane filter (0.45 µm) was then analyzed directly by RP-HPLC under the following conditions: flow rate 1 mL/min at 30°C, solvent A, water/acetic acid (97.5:2.5); solvent B, acetonitrile /water/acetic acid (80:17.5: 2.5), elution with linear-gradient (Table 1) followed by washing the re-equilibration, detection UV 280 nm. Each part of grape (skin and seeds) was extracted in duplicate and the acetonic fractions were analyzed in duplicate too. Hence, the final result was the arithmetic average of four analyses. The compounds identified in the seeds and Cardinal grape skin are listed in Table 2 (20 – 26) and Figure 1 shows the chromatographic profiles of identified tannins eluted according to their retention time.

Table 1: Linear gradient used for the separation of flavan-3-ols

Time (min)	% A	% B
0	95	5
3,4	88,5	11,5
5	80	20
23	50	50
25	40	60
28	5	95
32	5	95
35	95	5
38	95	5

2.5. Statistical analysis

Results are expressed as mean ± standard deviation (SD). Statistical analysis was carried out using Statistica software version 5.0 (Stat Soft, France). Differences between means were first analyzed using the ANOVA test, and the least significant differences (Fisher's LSD) were calculated following a significant *F* test ($p \leq 0.05$).

3. RESULTS

The total amount of PAs (Table 3), ranging from 537.25±35.28 mg/g to 1332.90±95.88 mg/g of berries for skin and seed, respectively. As expected, the seed PAs content is higher than in the skin, which is in accordance with the previous studies [6-8].

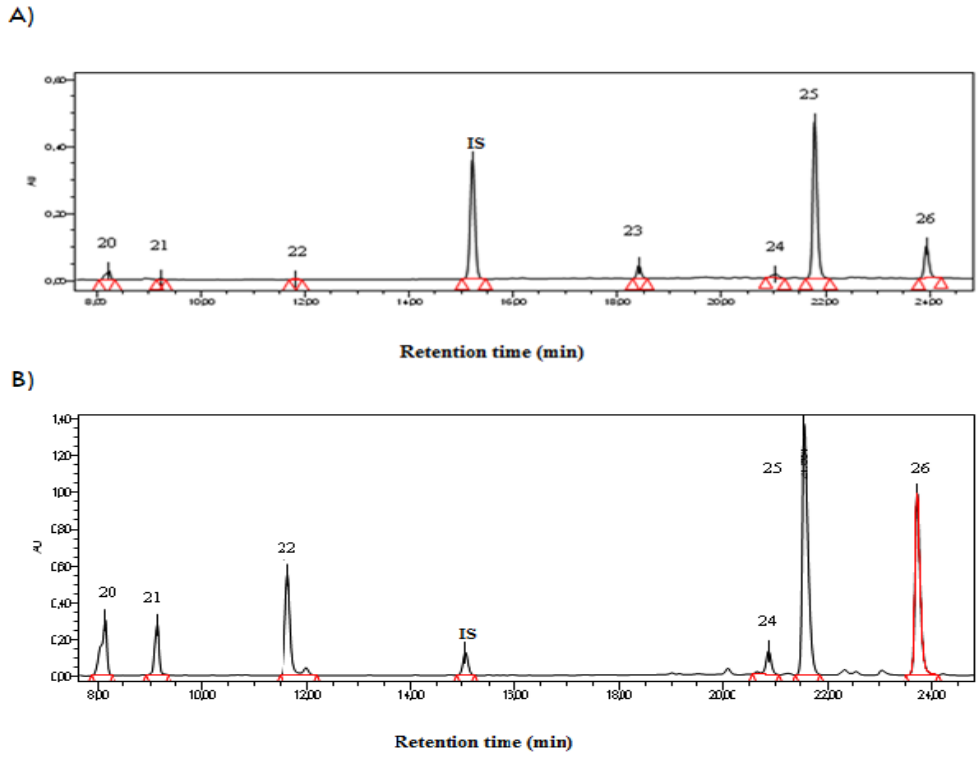

Figure 1: Proanthocyanidin chromatographic pattern of Cardinal skin (A) and seed (B) extract recorded at 280 nm. For key to substances, refer to Table 2

As shown in Table 3, the values obtained for the DPm; 16.33±1.19 for skin and 4.59±0.01 for seed respectively, show that seed tannins are in oligomeric and monomeric forms (DPm varies from 2 to 12 - 15) and skin PAs are in the polymeric form [7].

4. DISCUSSION

Regarding PAs contents, as published on two red grape varieties (Cabernet Sauvignon and Merlot), Lorrain *et al.* [9] recorded values varying from 90.1 ± 4.0 to 92.2 ± 4.5 in seed and from 57.4 ± 0.4 to 63.8 ± 0.1 mg/g on dry weight in skin. According to Brossaud *et al.* [6] on Cabernet Franc berries cultivated at different sites in the Loire Valley (France) - vintage 1995, the contents of PAs (condensed tannins) oscillated between 1.239 and 1.759 g / kg of the fresh weight and between 3.363 and 4.448 g / kg of the fresh weight in skins and seeds, respectively. These results are different from those found in our study. Those differences depend on the grape variety, environmental conditions, in particular water supply and sunlight exposure, berry size and number of seeds [10], variety and year of harvest [11], degree of maturation [12,13]. These differences also may highlight the impact of the different terroirs, the cultural practices, but also the grape harvest on

Table 2: Retention time of different identified proanthocyanidins in Cardinal skin and seed

	Proanthocyanidins	Retention time (min)
20	Catechin (C)	8.100 ± 0.079
21	Epicatechin (EC)	9.112 ± 0.075
22	Epicatechin-3-O-Gallate (ECG)	11.609 ± 0.129
EI	Internal standard (IS)	15.006 ± 0.127
23	Epigallocatechin-SH (EGC-SH)	18. 263 ± 0.129
24	Catechin-SH (C-SH)	20. 822 ± 0.136
25	Epicatechin-SH (EC-SH)	21.563 ± 0.134
26	Epicatechin-3-O-Gallate-SH (ECG-SH)	23.725 ± 0.128

Each value in the table is the mean ± standard deviation (n = 4).

Table 3: Proanthocyanidins, DPm, ECG, and EGC of Cardinal skin and seed

	Skin	Seed
Proanthocyanidins (mg/g of berries)	537.25±35.28	1332.90±95.88
DPm	16.33±1.19	4.59±0.01
ECG %	6.08 ± 0.0001	23.11 ± 0.004
EGC %	17.14±0.006	0.00 ±0.00

The results are expressed as mean ± standard deviation (n = 4); DPm: mean degree of polymerization; ECG: epicatechin gallate; EGC: epigallocatechin.

the metabolism path of the tannins [9]. According to Mateus *et al.* [14], the low altitudes appear to be favorable for the synthesis of important PAs concentrations in relation to climatic conditions, which coincide with high values recorded in this study for cardinal cultivated at very low altitude. Values of DPm were recorded on two varieties from Chili (Cabernet Sauvignon and Carménère) oscillated from 6.4±1.1 to 10.0±3.7 in skin and from 1.8±0.2 to 2.0±0.2 in seed, respectively for the two varieties [15]. The acid catalysis method used for fractionation of tannins may be, particularly, causing the differences recorded. According to Cadot *et al.* [16], homogenous polymerization of PAs during synthesis between fruit set and veraison increases astringency as their size increases, while combination with anthocyanins decreases the reactivity, and hence the astringency, of the compounds formed; So the astringency of cardinal variety directly depend on the DPm of its PAs. Table 3 results visibly show that skin PAs differ from those of seed by a lower percentage of galloylation (% ECG), high DPm and the presence of the prodelphinidin (EGC), as it was also observed by other authors for other varieties [1, 15,17].

5. CONCLUSION

The results of the present study show differences between total PAs, monomer PAs (ECG and EGC) and DPm of the two grape compartments (skin and seeds); but it remains that seeds and skin of Cardinal grape variety cultivated in this region of Algeria, are important sources of PAs known for their high antioxidant activity. This suggests their use as raw material for the extraction of these bioactive molecules, and their use, for example as specific additives in the food industry to replace chemical additives or as food supplements.

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Cite this article as: Benmeziane F and Cadot Y. Quantitative Analysis of Proanthocyanidins (Tannins) From Cardinal Grape (*Vitis vinifera*) Skin and Seed by RP-HPLC. *Nor. Afr. J. Food Nutr. Res.* July - December (2019);03(06):201-05. https://doi.org/10.5281/zenodo.3542124

The North African Journal of Food and Nutrition Research: (2019)03;(06):206-13
https://doi.org/10.5281/zenodo.3560326

Original Article

OPEN ACCESS

Making Nutrition a Development Priority in Africa

eISSN: 2588-1582

Contents lists available at

Journal homepage: https://www.najfnr.org

Viability of Lactic Acid Bacteria in Different Components of *Ogi* with Anti diarrhoeagenic *E. coli* Activities

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ARTICLE INFO

Article history:
Received 03 August 2019
Accepted 27 November 2019
Published 16 December 2019

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Access this article online

Quick Response Code

https://doi.org/10.5281/zenodo.3560326

Article edited by:
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ABSTRACT

BACKGROUND: *Ogi* constitutes a rich source of lactic acid bacteria (LAB) with associated health benefits to humans through antimicrobial activities. However, the high viability of LAB in *Ogi* and its supernatant (*Omidun*) is essential. **AIMS**: This study was carried out to assess the viability of LAB in various forms of modified and natural *Ogi* and the antimicrobial properties of *Omidun* against diarrhoeagenic *E coli*. **METHODS AND MATERIAL**: The viability of LAB was assessed in fermented *Ogi* slurry and *Omidun* for one month and also freeze-dried *Ogi* with and without added bacterial strains for two months. A further 10 days viability study of modified *Omidun,* refrigerated *Omidun,* and normal *Ogi* was performed. The antimicrobial effects of modified O*midun* against five selected strains of diarrhoeagenic *E. coli* (DEC) were evaluated by the co-culture method. **RESULTS**: Both drying methods significantly affected carotenoids and phenolic compounds. The *Ogi* slurry had viable LAB only for 10 days after which, there was a succession of fungi and yeast. *Omidun* showed 2 $\log_{10}$cfu/ml reduction of LAB count each week and the freeze-dried *Ogi* showed progressive reduction in viability. Refrigerated *Omidun* has little viable LAB, while higher viability was seen in modified *Omidun* (≥2 log cfu/ml) than normal *Omidun*. Modified *Omidun* intervention led to 2-4 log reduction in diarrhoeagenic *E. coli* strains and total inactivation of shigella-toxin producing *E. coli* H66D strain in co-culture. **CONCLUSIONS**: The consumption of *Ogi* should be within 10 days of milling using modified *Omidun*. There are practical potentials of consumption of *Omidun* in destroying *E. coli* strains implicated in diarrhea.

KEYWORDS: *Ogi, Omidun*, lactic acid bacteria, diarrhoeagenic *Escherichia coli* strains, Viability.

1. INTRODUCTION

Fermentation preserves foods by converting carbohydrates to alcohol and organic acids [1]. Microbes such as lactic acid bacteria (LAB) are involved in natural fermentation processes that produce fermented foods. [2,3]. The influences of the fermentation microbes on the nature of the food and their antimicrobial properties are well characterized [4-6]. Aderiye *et al.*, [7] described the use of fermented cereals as foods with enhanced health properties e.g. hypolipidemic, hepatoprotective, antibacterial, and treatment of gastroenteritis in man and animals.

WHO [8] described probiotics as "*live microorganisms which when administered in adequate amounts confer a health benefit on the host*" i.e. viability and consumption of sufficient numbers are an inherent property of probiotics. One of the best uses for probiotics is the reduction of infectious diarrhea and diarrhea associated with antibiotic use. Probiotics shorten diarrhea episodes. Diarrhoeagenic *Escherichia coli* strains are among the commonest causative agents of diarrhea and are divided into enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enterohaemorrhagic *E. coli* (EHEC), enteroinvasive

E. coli (EIEC), and enteroaggregative *E. coli* (EAEC). Some developing countries, such as Nigeria, are still struggling against increasing morbidity and mortality of diarrheal infections in young children. Different home remedies are usually employed to combat diarrhea menace. The use of *Omidun* (the supernatant on *Ogi*) constitutes an example. *Ogi* is a fermented cereal gruel widely consumed in Western Nigeria in breakfast and in traditional infant weaning food [9]. Aderiye and Laleye, [10] stated that, although some communities in south-western Nigeria administered uncooked O*gi* to people with diarrhea to reduce the frequency of stooling, the scientific proof for this claim is lacking. Several authors have described functional, nutritional and antibacterial properties of Ogi [11-15], but there is insufficient data on the viability of LAB in different components of *Ogi* over a period of time. Therefore, this study was designed to study the component of *Ogi* that has the most viable LAB over a period of time and the antimicrobial properties of *Omidun* against different strains of diarrhoeagenic *Escherichia coli*.

2. MATERIAL AND METHODS

2.1. Bacterial strains

2.1.1. Diarrhoeagenic *E. coli* strains

All diarrhoeagenic strains of *E. coli* were obtained from the Molecular Microbiology Unit, Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Nigeria. Five different strains of *Escherichia coli* (Enterohaemorrhagic *E. coli* (EAEC LLD25D), Enterotoxigenic *E. coli* (ETEC LWD21A), Shiga-toxin producing *E. coli* (STEC LLH74B), Enteroinvasive *E. coli* (EIEC LWD21E) and Enteropathogenic *E. coli* (EPEC LLH78D) were used for the modified *Omidun* co-culture experiment.

2.1.2. Lactobacilli strains

Two strains of already characterized LAB; *Lactobacillus paraplantarum* AI3, and *Lactobacillus pentosus* AO82 with good antimicrobial properties were obtained from the probiotic group of Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria.

2.2. Traditional fermentation of *Ogi*

Maize grains (*Zea mays*) were obtained from the Bodija market, Ibadan Oyo State Nigeria. The *Ogi* was prepared by fermentation of maize grains according to the traditional methods of processing as described by Afolayan *et al.* [14]. In summary, Maize grains were soaked in clean water for 48 h at 28 ± 2°C. The fermenting water was decanted and the soften maize grains were wet milled using clean grinding machine. The paste obtained was sieved with a clean muslin cloth to remove the husks. The filtrate was allowed to settle and ferment according to the days subsequently described for each experiment. The filtrate separated into thick paste (*Ogi*) and watery supernatant (Omidun). The pH was evaluated for a period of 7 days.

2.3. *Ogi* and *Omidun* viability studies

On the first day of souring, 1g of *Ogi* slurry was obtained from the surface of Ogi (this involves lightly scrapping the surface of settled *Ogi* in order to obtain maximum bacterial counts) and 1 ml of *Omidun* were appropriately diluted with 0.9% sterile normal saline and the dilutions were inoculated on MRS agar respectively [14]. All incubations were carried under micro-aerophilic conditions at 37°C for 24-48 hours. The number of colony-forming units on the MRS agar were counted and identified by morphological characteristics, Gram reaction, and catalase test. The procedure was repeated weekly on *Ogi* slurry and *Omidun* for 26 days.

2.4. Freeze-dried *Ogi* powder viability study

The effect of freeze-drying on LAB viability in *Omidun* and milk was done according to a modified method of Ayeni *et al* [16]. Grown 24 h cultures of *Lactobacillus paraplantarum* AI3 and *Lactobacillus pentosus* AO82 respectively were centrifuged at 3000 rpm for 10 mins, washed in normal saline and then resuspended in 0.5 mL *Omidun* and sterile milk respectively. The resuspended pellets of the LAB in *Omidun* was mixed with 10 g of wet Ogi slurry. Also the resuspended LAB in milk was mixed with 1 mL of milk for each sample. The viability counts of the mixtures were carried out before freeze-drying. All the five different components (*Ogi* alone, *Ogi + L. paraplantarum*, *Ogi + L. pentosus*, milk + *L. paraplantarum*, milk + *L pentosus*) were collected and freeze-dried by freezing them to -20°C at atmospheric pressure then sublimed the frozen product at -20°C, which was then transferred to a condenser at low temperature and then defrost to yield a powdered product. The viability counts before and after freeze-drying and also after 69 days of storage at room temperature was performed for bacterial strains vehiculated in *Ogi* and milk. For *Ogi,* the viability at 26 days was measured and then discontinued due to low survival rate of LAB.

A study on the effects of capsulation on lactobacilli vehiculated in *Ogi* was adapted from the viability count method of Ayeni *et al* [16]. Freeze-dried Ogi and milk were put in capsule shells, filled and spread over to ensure uniform filling of the capsules. The cap was fixed appropriately over the body and stored at room temperature. The viability counts of LAB in capsules versus the freeze-dried products that were not stored in capsules for the *Ogi* products were done after three weeks of

storage and the results recorded. The difference in the viability of LAB cryopreserved with milk *vs* Ogi was analyzed with student t-test.

2.5. Evaluation of the viability of LAB in *Ogi* over a period of ten days

The results obtained from the initially described viability study made us develop a new protocol to evaluate the maximum viability of Ogi components over 10 days. Freshly prepared *Ogi* with *Omidun* [14] were divided into three sterile containers. In the first container, *Omidun* was changed every day and viability study was done with mixture of *Omidun* (removed before changing the water) and lightly scrapped surface of *Ogi*. This mixture is tagged `modified *Omidun* ` and 1 ml of the mixture was serially diluted and plated out on MRS agar for viability counts after incubating micro aerophilically for 24-48 h. The procedure was repeated daily for 10 days. In the second container, *Ogi* was allowed to settle and *Omidun* changed daily. The surface of settled Ogi was lightly scrapped and 1 g obtained from the scrapped material was mixed in 9 mL of saline and diluted appropriately, then plated on MRS agar to get the viability counts daily for ten days. From the third container, *Omidun* was decanted after milling and settling, then kept in the fridge for 10 days. Analyses were done by daily removing 1 mL of the refrigerated *Omidun* and plating out as previously described for ten days.

2.6. Determination of the antimicrobial effect of modified *Omidun* on diarrhoeagenic *Escherichia coli*

The method of Ojo *et al*. [5] was used to study the antimicrobial effects of *Omidun* on diarrheagenic *E. coli* pathotypes. We used five different diarrheagenic *E. coli* strains: EAEC LLD25D, ETEC LWD21A, STEC LLH74B, EIEC LWD21E and EPEC LLH78D. The strains were grown for 24 h in Nutrient Broth and 0.1 mL of each *E. coli* strains were introduced into 99.9 mL of modified *Omidun* mixture as previously described. One milliliter from the mixture was diluted serially in 9 mL of normal saline and plated out on MacConkey agar to get the viable counts of the *E. coli* strains at time zero (0 h) by incubating for 24 h at 37°C. The remaining 99 mL mixture of modified *Omidun* and *E. coli* were incubated for 24 h at 37°C aerobically. One milliliter from the mixture was diluted serially in 9 mL of normal saline and plated out on MacConkey agar to get the viable counts of the *E. coli* strains at time 24 (24 h) by incubating for 24 h at 37°C. The control experiment involved growing the different *E. coli* strains in normal saline and plating out the viable cells at time 0 h and 24 h. The plates were then observed for the growth of *E. coli* and viable colonies counted. The results were recorded at 0 h and 24 h.

3. RESULTS

The pH of traditionally prepared *Ogi* was evaluated over 7 days as Day 1: 3.96, Day 2: 3.45, Day 3: 3.77, Day 4: 3.62, Day 5: 3.50 - Day 7: 3.98. The highest pH was 3.98 on day 7 and the lowest was 3.45 on day 2. The result of quantities of viable LAB in different components of *Ogi*: *Ogi* slurry; *Omidun,* modified *Omidun* and freeze-dried *Ogi* at different time intervals are shown on Figures 1 to 3. There was an increase in quantity of viable LAB in *Ogi* slurry as the number of days increases, ranging from 8.6×10^8 cfu/ml on day 1 of the souring period to one log increase (5.2×10^9 cfu/ml) on day 3 and further one log increase (9.7×10^9 cfu/ml) on day 10. However, after ten days, there was succession of fungi growth (Fig 1). The LAB present in *Omidun* showed viability for the four weeks duration, though with progressive reduction in quantities of viable LAB as the days from 1.25×10^7cfu/ml on day 1 to 3.5×10^2 cfu/ml on day 27 (Fig. 1).

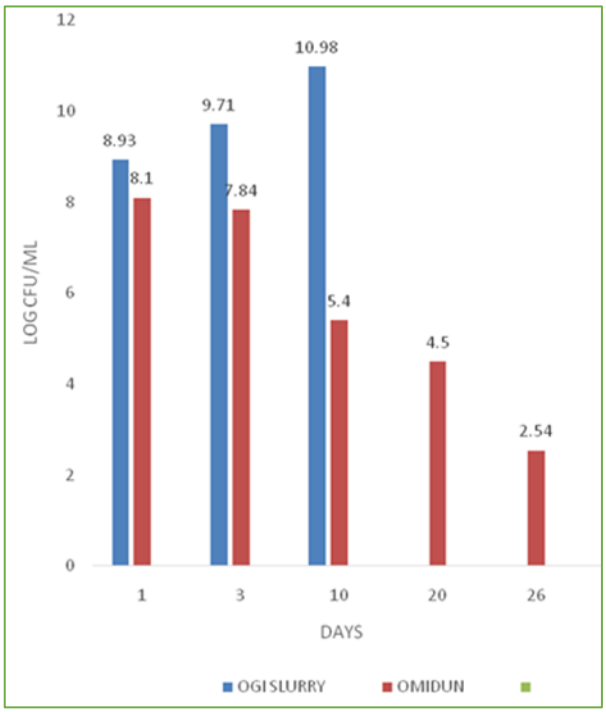

Figure 1: Viability of LAB in *Ogi* slurry and *Omidun*

In the 10 days viability study, LAB in *Omidun* remained viable over a period of 10 days. On the first day, modified *Omidun* had LAB counts of 6.2 x 10^7 CFU/ml, *Ogi* alone had 3.5 x 10^9 cfu/ml and refrigerated *Omidun* had 1.0 x 10^8. Maximum counts were recorded on the fifth day as 2.4 x 10^9 cfu/ml for modified *Omidun*, 7.2 x 10^9 cfu/ml for raw *Ogi* and refrigerated *Omidun* had 2.0 x 10^6 cfu/ml. Over the fifth day, there was a decline in the counts of LAB in all the three fractions of *Ogi* used (Fig. 2).

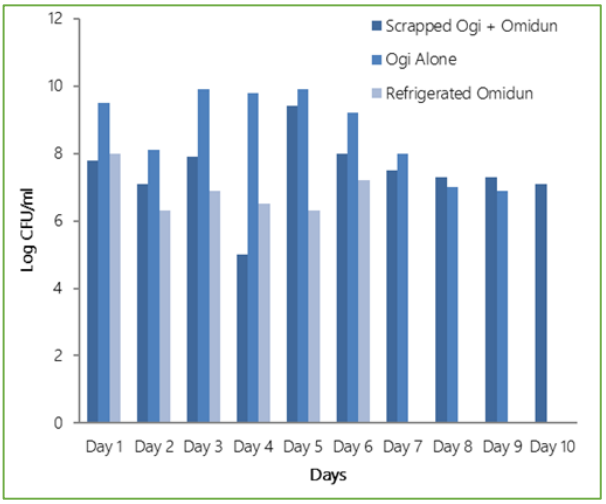

Figure 2: Viability of LAB in modified *Omidun*, refrigerated *Omidun*, and *Ogi* for 10 days

The effect of freeze-drying on the viability of *L. pentosus* and *L. paraplantarum* using *Ogi* and milk as cryo-protectants was reported on Fig. 3. Milk is a better cryopreserving agent than *Ogi*. For LAB strains cryopreserved in *Ogi*, there was a 3 log reduction in the cfu/ml after freeze-drying in both tested strains while for LAB strains cryopreserved in milk, there was only a slight reduction in the viability of two LAB, from 2.6 x 10^{12} to 1.1 x 10^{12} for *L. paraplantarum* and from 2.32 x 10^{12} to 6 x 10^{11}.for *L. pentosus*. In freeze-dried *Ogi,* the reduction in viable cells was four logs from 5.2 x 10^{9} to 7.2 x 10^{5} cfu/ml (cf. Fig 3). Also, the effect of two months of storage at room temperature, on the viability of freeze-dried *L. pentosus* and *L. paraplantarum* cryopreserved in *Ogi,* milk, and *Ogi* alone, was reported. The viable LAB in *Ogi* alone reduced from 7.2 × 10^{5}cfu/ml to 1.3×10^{2} cfu/ml on day 26. There was two log reduction in the viability of *L. pentosus* cryopreserved with *Ogi* (not preserved in capsules) between day 1 and day 69 (from 2.77 x 10^{8} to 2.9 x 10^{6}cfu/ml) while there was a 3-log reduction in the viability of *L. paraplantarum* cryopreserved with *Ogi* (not preserved in capsules) (from 3.04 x 10^{8} to 1.32 x 10^{5}.cfu/ml) (cf. Fig. 3). There was a statistically significant difference in the viability of LAB cryopreserved with milk versus *Ogi* (p=0.012).

The effect of 3 weeks of capsulation on freeze-dried strains cryopreserved in *Ogi* was reported. There was a drastic reduction in the viability of *Ogi* capsulated products at the end of 3 weeks to <10^{4} cfu/ml in both strains, thereby leading to discontinuation of the experiment. However, the uncapsulated *Ogi* freeze-dried *L. paraplantarum* strain had a three-log reduction from 3.04 x 10^{8} cfu/ml to 1.62 x 10^{5} cfu/ml and a one log reduction for *L. pentosus* (from 2.77 x 10^{8} to 7 x 10^{7} cfu/ml) (cf. Fig. 4). The milk capsulated products survived till the 69th day, but at reduced viability.

For milk+ *L. paraplantarum*, there was a 6 log reduction (from 1.1 x 10^{12} to 3.7x10^{5}) and for milk+ *L. pentosus*, there was a 5 log reduction (from 6 x 10^{11} to 1 x 10^{6}) after 69 days (cf. Fig. 3).

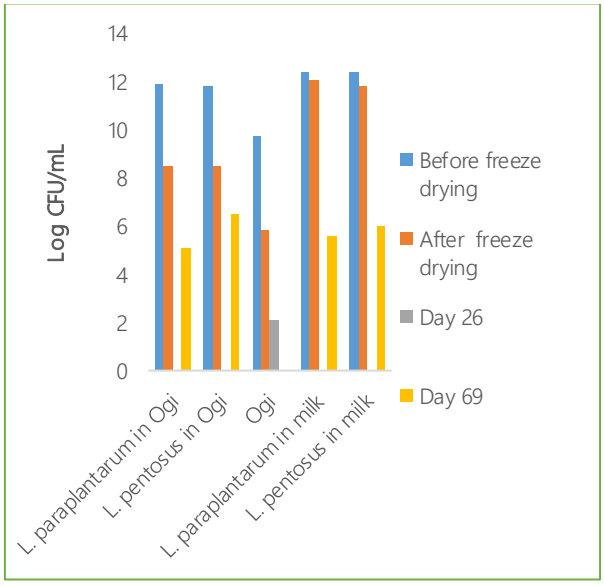

Figure 3: Effects of freeze-drying and storage on Lactobacilli strains

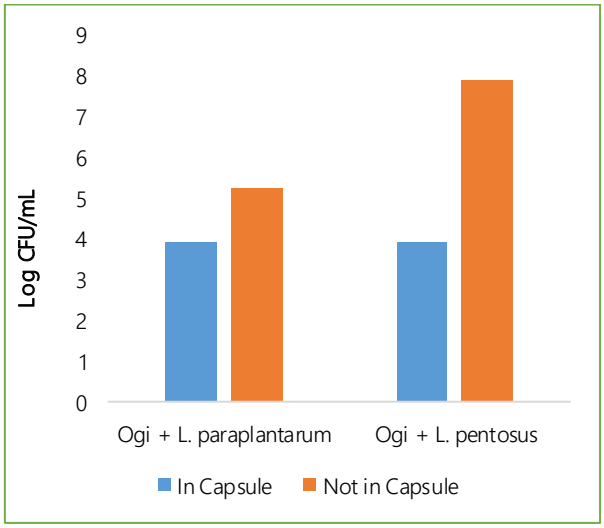

Figure 4: Comparison of the viability of Lactobacilli strains in freeze-dried *Ogi* after three weeks of capsulation and uncapsulation

The antimicrobial effect of modified *Omidun* against five strains of diarrhoeagenic *E. coli* was reported. For the control experiments, the *E. coli* strains either maintain, lower or increased its viability after incubation in saline for 24 h e.g. the log viability of EAEC (3 logs), EHEC (5 logs) and STEC (6 logs) were maintained at 0 and 24 h, EPEC increased from 4 logs to 5 logs while ETEC reduced from 6 to 5 log count. STEC was the most susceptible *E. coli* strain as no strain survived in *Omidun* after 24 hours of

incubation. There was a 7 log reduction in ETEC strain, 4 log reduction in EAEC and 2 log reduction in EIEC. *Omidun* had little effect in reducing EPEC viability (cf. Fig 5).

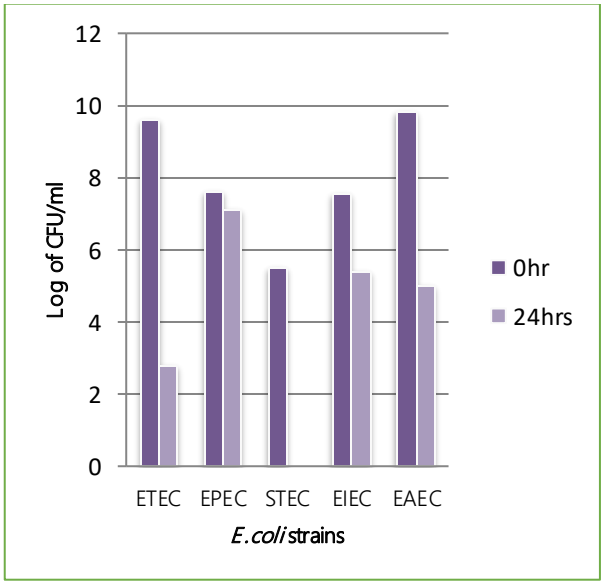

Figure 5: Antimicrobial Effects of Modified *Omidun* on Diarrheagenic *E. coli* strains

4. DISCUSSION

This study reported that scrapping the surface of *Ogi* and mixing it with *Omidun* had a higher quantity of viable beneficial LAB in comparison to normal *Omidun* and freeze-dried *Ogi* with appropriate anti diarrheagenic *E. coli* activities thereby implying functional food ability of *Ogi.* We also report complete absence of LAB in *Ogi* after 10 days of milling, but rather a succession of fungi and yeast, thereby suggesting that the shelf life of *Ogi* is within 10 days if they are to be used as a functional food. *Ogi* could be consumed strictly as food with no consideration for attendant health benefits. However, if *Ogi* is to be considered as functional food with special interest in the naturally occurring beneficial bacteria, then, the viability of the bacteria is significant as probiotics, defined as live bacteria which when administered in adequate amount confer health benefits on the host. Therefore, viability of bacteria in functional food constitutes a key consideration.

The *Ogi* slurry used in the current study showed a progressive increase in the LAB population for a duration of 10 days after which there was a succession of fungi with no viable LAB. A repeated experiment confirmed that the viability of LAB in *Ogi* is only within 10 days. The occurrence of LAB in *Omidun* was reported by George and Anosike [17], where viable LAB was isolated from *Omidun* and the increase in population of LAB is supported by Afolayan *et al.*, [14] where the LAB growth in the *Ogi* slurry increased during 48 h of the souring period. However, to the best of our knowledge, this is the first study reporting a 10 days period for detecting viable LAB in *Ogi.*

The counts of LAB in the modified *Omidun* and *Ogi* were more than that of refrigerated *Omidun* with peak viability on the fifth day. This can possibly be attributed to fermentation attaining its peak on the fifth day. Subsequently, there was a decline in the count of LAB since it was assumed that the LAB were the major agents of fermentation. The lower counts of LAB recorded for refrigerated *Omidun* may have been due to the fact that refrigeration prevented fermentation from occurring. This is in contrast with the results reported by Afolayan *et al.*, [14] who recorded higher counts of LAB in *Omidun* than in raw *Ogi* and this may be due to the fact that the *Omidun* in their study was stored at room temperature which was not the case in this study. There have been reported of peak LAB count and after which, there was a decline in the counts of the viable LAB [14,18]. These findings are indicative of the fact that the normal preparation of *Ogi* or a mixture of raw *Ogi* and *Omidun* contain high quantities of LAB until the fifth day when stored at room temperature and the water is constantly changed.

The reduction in LAB counts in *Omidun* and increases in *Ogi* slurry can be attributed to the gravitational pull of the LAB from the *Omidun* to the *Ogi* surface. Therefore, a modified *Omidun,* used in our study, involves the scrapped surface of *Ogi* mixed with *Omidun* and it displays a high count of viable LAB. The modified *Omidun* viable count was higher than traditional *Omidun* because it combines viable counts in *Omidun* with the densely populated surface of *Ogi* where gravitational force has pulled down the bacteria. Aiming to provide health benefits, it will be essential that there is a minimum of 10^6 cfu g^{-1} viable probiotic organisms in a product [14] or 10^7 cfu g^{-1} at point of delivery [19]. Therefore, observed high viability of LAB in different components of *Ogi* is interesting.

In formulating freeze-dried products, the cryoprotectant has to be considered and it is essential that viability is maintained throughout the process of formulation and subsequently throughout its use. During freeze-drying, the cells are exposed to an extreme temperature that has the ability to damage the cells of the bacteria [20]. Cryoprotectants can then be used in optimizing this process, protect the cells and in the process enhance the viability of the organisms during freeze-drying [20]. As observed in this study, *Ogi* is not a suitable cryoprotectant during freeze-drying and capsule storage in comparison to using skimmed milk. The LAB, in formulation with milk, possesses a higher survival rate than LAB in *Ogi* formulation. Ayeni *et al.* [16] reported that milk is highly effective in protecting the organisms during freeze-drying

and enhancing the survival of the organisms during storage. This agrees with the study carried out by Jalali *et al.* [20] who reported 20% increase in viability of the organisms using 6% skimmed milk and the highest survival after 3 months in capsules that formulation is with sodium ascorbate and trehalose. Freeze-dried techniques have the advantage of being a process in which bacteria can survive well with the addition of cryoprotectants [16,20,21]. Temperature fluctuation is one of the factors that contribute to the survival and activity of LAB in a food sample [22]. The freeze-dried process has the advantage of preserving the LAB for a long time and also reduces the rate of destruction by the gastric acid due to the ease of micro-encapsulating a freeze-dried product.

Escherichia coli, which has been implicated as one of the major causes of diarrhea in humans, and the major cause of mortality and morbidity in children less than 5 years, has shown multi-resistance to antibiotics. The resistance of diarrhoeagenic *E. coli* to antibiotics has been ascribed to the misuse or under-use of antibiotics most especially ampicillin, chloramphenicol and sulphamethoxazole-trimetroprim [23,24]. Fermented foods can have an inhibitory effect on the diarrhoeagenic *E. coli* which could be due to different mechanisms of action of the LAB present in the fermented food [5,25]. These inhibit the growth of the pathogenic organism and the inhibitory effect is supported by a decrease in pH hence increase in acidity of the environment. From the co-culture experiment, there was reduction in the viable count of the selected diarrhoeagenic *E. coli* strain after 24 h of contact time. Interestingly, after 24 h, STEC LLH74B was entirely inhibited and drastic inhibition was observed with the other diarrhoeagenic *E. coli* strains. Afolayan and Ayeni [26] also observed a decrease in the count of *E. coli* strain EKT004 after a co-culture with LAB isolated from *Ogi* with more inhibitory activity of LAB against *E. coli* strain EKT004 when compared with the activity of conventional antibiotics. These reports demonstrate antimicrobial activity of LAB in fermented food, especially *Omidun* which in turn suggests that this group of bacteria is able to confer health benefits on individuals consuming them. It may, therefore, be important to encourage the use of a mixture of *Omidun* and raw *Ogi* or raw *Ogi* for better results.

The observed activities could be due to inhibitory compounds produced by lactobacilli e.g. organic acids, diacetyl, hydrogen peroxide, nisin, lactic acid and bacteriocins [25,27,28]. George and Anosike [17] also isolated three LAB from *Omidun* and showed their antimicrobial effect on some test microorganisms and Ayeni and Ayeni, [29] reported that inoculated enteric pathogen was inhibited by LAB after 24 - 48 h contact time. Furthermore, the decrease in the pH contributes to the inhibitory effect of the *Omidun* on *E. coli.* There was a drastic change in the pH value with a decrease from 4.06 to 2.90, hence an increase in acidity. This drastic change in pH has been reported [14]. The effectiveness of *Lactobacillus* species against enteropathogenic bacteria has been reported [30,31]. Interestingly, *Omidun* doesn't only have antimicrobial properties, but it offers protection against colitis in a rat model [32] . This further clarifies the medicinal properties of *Omidun* as reported in our study.

5. CONCLUSION

The current study provides the scientific proof of the use of *Omidun* in the local treatment of diarrhoea due to its anti-diarrhoeagenic activities. Furthermore, we demonstrated that *Ogi* and *Omidun* are best consumed within 10 days of souring for maximal lactic acid bacterial viability and antimicrobial effects. We present a modified *Omidun* that involves lightly scrapping the surface of *Ogi* and mixing it with *Omidun* to get a higher quantity of viable beneficial LAB.

Acknowledgment

We acknowledge Prof. Iruka Okeke of Molecular Microbiology Unit, Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria for the provision of diarrhoeagenic *E. coli* strains used in this study.

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Cite this article as: Kwasi RE, Aremu IG, Dosunmu QO, Ayeni FA. Viability of Lactic Acid Bacteria in Different Components of *Ogi* with Anti diarrhoeagenic *E. coli* Activities. *Nor. Afr. J. Food Nutr. Res.* July - December (2019);03(06):206-13. https://doi.org/10.5281/zenodo.3560326

The North African Journal of Food and Nutrition Research: (2019)03;(06):214-18
https://doi.org/10.5281/zenodo.3571814

Short Communication

OPEN ACCESS

Making Nutrition a Development Priority in Africa

eISSN: 2588-1582

Contents lists available at

Journal homepage: https://www.najfnr.org

Development of Ready to Serve Pineapple Juice with Coconut Milk

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ARTICLE INFO

Article history:
Received 01 July 2019
Accepted 06 December 2019
Published 18 December 2019

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Access this article online

Quick Response Code

https://doi.org/10.5281/zenodo.3571814

Article edited by:
Dr. Sofiane DAIRI
Dr. Omar AOUN

ABSTRACT

BACKGROUND: Being available in Sri Lanka, Ready-to-serve drinks are more popular among Sri Lankan' consumers . Further, ready-to-serve organic fruit drinks are nowadays becoming more popular due to more concern about healthy living. **AIM:** To produce a ready-to-serve drink using pineapple juice with coconut milk. **METHODS AND MATERIAL**: Pineapple Juice (*Ananas comosus*) and Coconut milk were optimized to a blended ready to serve beverage which was mixed in four different predetermined ratios and stored for 14 days in glass bottles (200ml capacity). Physicochemical and sensory analysis were done according to the standards procedures. After 14 days of incubation period, four samples were tested for their sensory properties at CBL Natural foods laboratory. **RESULTS**: The ratio of pineapple juice: coconut milk (71:29) was ranked as highest score (106) for sensory evaluation and content Total suspended solids (13 °Brix), pH (4.25) and moisture (82.32). **CONCLUSIONS**: The formulation of mixed blend Pineapple juice beverage is possible to satisfy consumer tastes and preferences.

KEYWORDS: Ready-to-serve drink, Pineapple juice, Coconut milk, Physicochemical, Sensory properties.

1. INTRODUCTION

Several worldwide products are commercially accessible as ready-to-serve foods. A relatively simple and understandable interpretation to define ready-to-serve foods as prepared complete meals or menu items intended for sale other than on the premises where they were produced, primarily in retail stores, supermarkets or restaurants [1]. Ready-to-serve drinks are available all over the world even in Sri Lanka where they are more popular. Furthermore, ready-to-serve organic fruit drinks are currently becoming more popular due to more concern about healthy life. Pineapple (*Ananas comosus*) constitutes one of the most popular fruits of the non-citrus group tropical and subtropical fruits due to its attractive flavor and refreshing sugar-acid balance [2]. On the other hand, pineapple fruit contains high amounts of macro and micronutrients especially minerals and vitamins. Pineapple juice is frequently consumed around the globe, commonly as a canning industry, in the form of single strength, concentrated and in the blend composition to obtain new flavors in beverages and further products [3]. Popularly enjoyed in Sri Lanka as fresh fruit by the local consumer to fulfill their nutrient requirement and preference. In this country, the total extent of pineapple cultivation is about 4,750 hectares for producing a total of 35,000mt/year and production has progressively increased [4]. The fruit is available throughout the year and in some seasons, it is

under-utilized fruit. However, more waste is generating in industrial scale production which can be used for further processing.

Coconut (*Cocos nucifera*), is however a distinguished widely consumed fruit containing some amount of sweet water inside. This fruit represents an excellent source of minerals, such as: potassium, copper, calcium, iron, magnesium, and zinc. B-complex vitamins are also found in coconut. In addition, some saturated fatty acids could be available, as lauric acid that can prevent atherosclerosis. Moreover, coconut being one of the multipurpose and vital food items for millions of inhabitants of the south and south-east Asia and the Pacific islands, it constitutes one of the most sought-after ingredients, when mixed in most recipes prepared in Sri Lanka [5]. Coconut milk is a milky liquid obtained by manual or mechanical extraction of fresh coconut kernel with or without the addition of water. This extracted liquid is a white opaque protein-oil-water emulsion [6] and defined as "functional food" since it's not only providing basic nutrients but other health benefits. A highest per capita consumption of coconut milk was recorded from Sri Lanka (30 to 36 kg) [7].

Fresh juices contain antioxidants, minerals, and vitamins that are essential to promote a healthy life among consumers and preventing several diseases. Juice making is one of the best techniques to improve the nutritional quality of several types of foods and can improve the vitamin and mineral content depending on the kind and quality of fruits used [8]. Apart from nutritional quality improvement, blended juice can be served as appetizers and making a new product as a ready-to-serve drink is timely important since they are more popular among consumers.

Therefore, the present study aimed to produce pineapple juice with coconut milk since its richness of constant nutritional content. Coconut milk is a common supplementary beverage used by many Sri Lankans primarily in villages. Organic pine-coco milk is ready to drink natural fruit beverage with no added additives, preservatives, sweetening and coloring agents. This drink is 100% natural product and an appropriate source for both macro and micronutrients constituting a nutrient-dense fruit drink. In this introductory paper, we attempted to define this item and production technologies. However, the quality aspect of the product will be briefly discussed.

2. MATERIAL AND METHODS

The fully matured, freshly harvested pineapple fruits were obtained from the local market Sri Lanka and were brought to the CBL Natural Foods (pvt) ltd.

2.1. Pineapple juice preparation

Pineapple fruits were washed with potable water and outer peel was removed. Pineapple second peel and pineapple core were collected and underwent a crushing process. Then, crushed pineapples were feed to a pulping machine to get pineapple juice. After that, separated pineapple juice was filtered throughout vibration sieving machine (110 microns). Subsequently, clear pineapple juice was pasteurized at 80 ºC for 5 minutes and transferred to a stainless steel container. Total Soluble Solids (TSS) (ºBrix) and pH values were measured accordingly.

2.2. Coconut Milk preparation

Previously prepared sterilized coconut milk cans were used for the preparation of ready-to-serve fruit drinks.

2.3. Preparation of ready-to-serve fruit drink

Prepared natural pineapple juice and coconut milk were mixed in different ratios as summarized in Table 1. The mixture was then filled into clean, sterilized normal glass bottles and sealed manually. Afterward, sealed bottles were sterilized under 110 ºC and 1 bar for 60 minutes (Steritec Automation Sterilizer). Prepared samples were kept at room temperature (29 ºC) for 14 days (Figures 1 and 2).

2.4. Sensory analysis

The present study aimed to collect the maximum possible quantity of coconut milk in the juice mixture with higher sensory scores and adjustment of acidity to provide a suitable taste. Sensory analysis was carried out at the level of quality assurance department in CBL Natural Foods (pvt) ltd. A panel of 30 trained testers aged between 18 to 50 years old carried out the acceptance tests. Each of the four different combinations (juices) were presented as a 40 ml sample in transparent glass cups and were evaluated by testers [9]. The test was performed by the rating option (rank 1; lowest acceptance to 4; highest acceptance), requested the panelists to rate overall quality including flavor. The samples were monadically served.

Figure 1: Prepared four different ready-to-serve fruit drinks

Table 1: TSS and pH values for different mixing ratios and final sample mixtures

Sample	Pineapple juice			Coconut milk			Juice Mixture	
	Quantity (g)	TSS (°Brix)	pH	Quantity (g)	TSS (°Brix)	pH	TSS (°Brix)	pH
01	3010	14	3.76	405	4	6.20	13	3.90
02	3010	15	3.75	810	4	6.20	14	3.92
03	3010	16	3.97	1215	4	6.20	12	4.10
04	3010	16	3.97	200	4	6.20	15	4.10

Table 2: Sensory scores for sensory evaluation of four combinations

Final products	01	02	03	04
Total score	70	64	106	61

2.5. Physicochemical Analysis

Total Soluble Solids (TSS) (°Brix) and pH values were measured with refracting photometer (ATAGO) and pH meter (EUTECH, Thermo Scientific) respectively. Moisture content (%) was measured with a moisture analyzer (MB 45, OHAUS) [10]. Each combination had three replicates and was measured for selected parameters and recorded average values.

2.6. Statistical Analyses

For physicochemical data including replicates tested and sensory evaluation data, one-way Analysis of Variance (ANOVA) served to determine whether significant differences ($p<0.05$) existed between four combinations of juice samples using MINITAB 15 version statistical software.

3. RESULTS AND DISCUSSION

3.1. Sensory evaluation

An observable sensory difference was observed between prepared pineapple juices. Where, the results of this study have shown that on an overall basis, consumers prefer the sensory characteristics of prepared pineapple juices in four different ways mix with coconut milk, without product information. These results can only be attributed to the sensory characteristics associated with the pineapple juices since no information was provided to influence preference.

Sensory scores for sensory evaluation of the four combinations were recorded in Table 2. The intention was to incorporate the maximum possible quantity of coconut milk in the juice mixture with higher sensory scores. Some researchers have shown that taste is the main driver for food selection, followed by health considerations [11,12]. However, Bower *et al.* [13] documented that, consumers were willing to pay more, and were more accepting of taste when the information was provided about the benefits of health and the price of the product. In the present study, it was observed that the highest sensory score (106) was obtained with maximum incorporation of 29% coconut milk in the juice mixture. Therefore, the ingredient compositions having 71% pineapple juice and 29% coconut milk were selected as optimum and used for storage study. Overall quality evaluation of statistical analysis showed a significant difference at a 95% confidence level (F-value > F-listed value) for four different juice mixers (cf. Electronic Supplementary Material).

As an overall evaluation, the average values of the sensory analysis were kept in the acceptance range. No alteration in the average values at the final storage period was found. Therefore, product acceptance can be considered good.

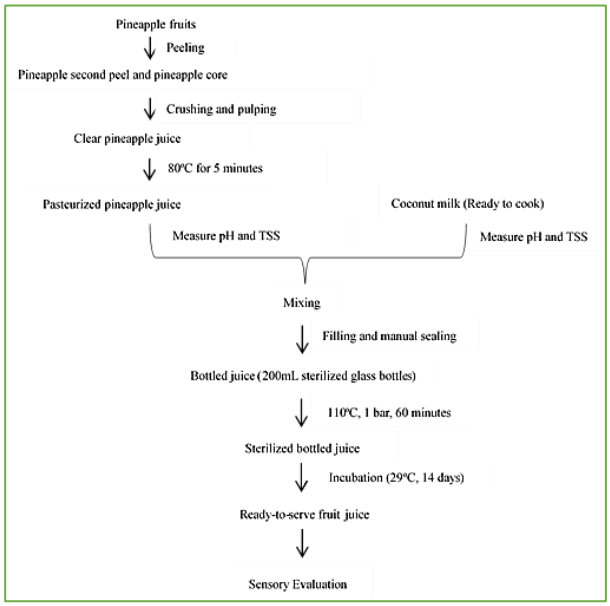

Figure 2: Processing flow chart

3.2. Physicochemical Analysis

pH was slightly increased in the final products except for the 4th sample (Tables 1 and 3). This might be due to the high percentage of Coconut milk. Decrease in pH was due to the increase in acidity of juice which affects the quality

of juice. It was noticed that coconut milk was a major factor to increase pH in the final product. Further, it was observed that the maximum pH (4.25) was recorded in the pineapple juice blended with coconut juice sample 03 which was taken the highest sensory score for sensory analysis. Statistical analysis of pH values showed a significant difference at a 95% confidence level (F-value > F-listed value) for four different juice mixers.

According to our results, sample number two was recorded the lowest TSS value (11 ^{0}Brix) where sample number four recorded the highest Brix value (15.6 ^{0}Brix). High Brix value may due to the high content of Pineapple juice in number four sample mixture. Further, the TSS was increased with the storage time; this may be due to the hydrolysis of polysaccharides into monosaccharides and oligosaccharides (Tables 1 and 3). Statistical analysis of ^{0}Brix values showed a significant difference at a 95% confidence level (F-value > F-listed value) for four different juice mixers.

Table 3: TSS, pH and moisture values in final sample mixtures

Sample category	Chemical parameter		
	TSS (ºBrix)	pH	Moisture content (%)
01	15.0	4.14	82.02
02	11.0	4.12	85.26
03	13.0	4.25	82.32
04	15.6	4.02	82.91

The highest moisture content was recorded from sample number two (85.26%) and statistical analysis of moisture content in juice mixtures were showed significant difference at 95% confidence level (F-value > F-listed value).

4. CONCLUSION

It was concluded that the ratio of pineapple juice: coconut milk (71:29) which was ranked as highest score for sensory evaluation and most effective juice blend for minimum change in TSS (13 ºBrix), pH (4.25) and moisture (82.32%). Therefore, the present study results revealed that the formulation of mixed blend juice beverage is possible to satisfy consumer taste and preferences.

Acknowledgment

The authors gratefully acknowledge the support of CBL Natural Foods (pvt) Ltd. Furthermore, the authors would like to thank Mrs. H.S.S. Fonseka, the Quality Assurance Manager and all officers of CBL Natural Foods (pvt) Ltd for their valuable assistance.

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Cite this article as: Kumara A, Mahagamage M.G.Y.L, and Arampath P.C. Development of Ready to Serve Pineapple Juice with Coconut Milk. *Nor. Afr. J. Food Nutr. Res.* July - December (2019);03(06):214-18. https://doi.org/10.5281/zenodo.3571814

The North African Journal of Food and Nutrition Research: (2019)03;(06):219-28
https://doi.org/10.5281/zenodo.3592037

Original Article

OPEN ACCESS

Making Nutrition a Development Priority in Africa

eISSN: 2588-1582

Contents lists available at:

Journal homepage: https://www.najfnr.org

Acceptance of salt reduction in bakery bread among Moroccan consumers

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ARTICLE INFO

Article history:
Received 08 September 2019
Accepted 21 December 2019
Published 12 January 2020

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Access this article online

Quick Response Code
https://doi.org/10.5281/zenodo.3592037

Article edited by:
Pr. Rekia BELAHSEN
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ABSTRACT

BACKGROUND: The use of cooking salt (sodium chloride) for bread preparation is due to several important sensory and technological properties. Even considered as an essential micronutrient and a salty taste stimulus, a significant dietary sodium intake is positively correlated with high blood pressure levels and some heart diseases. Recently, Morocco has adopted a plan to reduce salt consumption aiming to reinforce the prevention of Non-Communicable Diseases (NCDs) and to contribute to the achievement of 2025 global voluntary targets, set by the Second International Conference on Nutrition (ICN2). **AIMS**: The aim of the present study was to determine the acceptance of bakery bread with a different percentage reduction of salt by the Moroccan population. **SUBJECTS AND METHODS**: Various percentages of salt reduction in experimental bread; 7%, 10%, 16%, 23%, 30%, and 53%, were tasted and compared with standard market bread by 201 individuals. "Just About Right" (JAR) and purchase scales were utilized to score the different sample bread. **RESULTS**: Bread with 10% and 16% salt reduction were highly accepted by 76% and 79% of tasters, respectively. Based on the JAR score, these types of bread were considered as "just about right' by 50% and 57% of the participants respectively. The best average score of purchase intent was obtained for salt content of 1.62g and 1.56g per 100g for a reduction of 10% and 16% respectively. **CONCLUSIONS**: The current adopted strategy allows a 16% sodium reduction while maintaining taste quality.

KEYWORDS: Sodium chloride, bread, salt reduction, Moroccan population.

1. INTRODUCTION

Sodium occurs naturally in most foods such as animals, plants, and water. However, this mineral is excessively added to processed foods by manufacturers and by consumers during cooking or on the table during meals. Cereals and their products, especially bread and breakfast cereals, provide about 40% of average intake [1]. Therefore, bad eating habits particularly westernized diet pattern characterized by overconsumption of salty foods, constitute a major risk factor for developing NCDs spreading in several developing countries [2].

In the 18th century, salt was widely utilized for food preservation purposes with an average of 10 g / kg [3]. However, in the 20th century, the average salt content in bread doubled to reach 20 g / Kg. The most appropriate method to ameliorate taste, reduce the damage caused by

mold spoilage, and preserving foods, rather than salting, is to consider further alternatives [3,4].

The main source of sodium depends on the population's dietary habits [5]. Sodium is found in small quantities in a variety of natural products as well as processed foods (about 70 to 75% of daily intake) such as bread, crackers, and further manufactured products [6,7]. A food processed based diet, low in fresh fruits and a vegetable is considered as high in sodium [7]. Indeed, the diet pattern is related to the amount of salt and, therefore, the amount of sodium.

Bread, especially wholemeal bread, constitutes an essential source of complex carbohydrates, proteins, minerals, fats, and B-group vitamins [8]. In Morocco, bread is a staple of the Moroccan diet constituting a potential source of sodium. The daily consumption of bread can reach 500 g per person [9]. According to Derouiche *et al.* [10], the average quantity of salt added, for the preparation of white bread, was 17.42 ± 1.28 g/kg, being equivalent to a daily intake of 8 to 9 g of salt per bread. These amounts exceed the WHO's recommendations [9]. Thus, the high salt content of white bread could contribute to the increase of sodium intake within the population [11].

Sodium reduction in bakery products and processed foods would be beneficial for public health. Indeed, this nutrient, in its chloride form, should be moderately consumed according to dietary intake standards [12]. Sodium chloride can reduce microbial activity as a barrier to microbial growth and survival [13]. In addition to its unique taste, sodium chloride displays a strong effect on wheat gluten properties. Sodium chloride is an essential ingredient for the proper development of the bread dough structure. The interaction of salt with the flour components such as gluten is crucial to form high-quality breadcrumbs. Moreover, it guarantees satisfactory microbiological safety [14].

Hence, a significant decrease in salt will affect the functional and sensory properties of bread and may also reduce its acceptability by consumers. An adequate daily intake of salt (the equivalent of 180-230 mg of sodium) is essential for normal body functions [15]. However, a high dietary sodium intake is directly related to the development of NCDs such as hypertension, cardiovascular diseases, or coronary heart disease [16-18], representing the most significant pathologies causing global death [19]. In 2016, NCDs were responsible for 71% (41 million) of the 57 million deaths that occurred worldwide versus 60% of all deaths and 43% of disease burden in 2012 [18,20]. In this context, the most recent estimate on the daily intake of salt in Morocco was carried out by the Ministry of Health in 2008 prior to the addition of iodine to salt. This survey showed that salt intake among adults reached a daily consumption of 7–12 g/person [21]. This amount is exceeding the WHO's recommendations (< 5 g of salt a day eq. < 2 g of sodium per day) [18].

Concerning this high intake of salt, a well-studied plan has been established to increase awareness among the Moroccan population against overconsumption of salt and its harmful effects to prevent the emergence of NCDs. ICN2 projected a list of 60 recommendations, of which recommendation 14 encourages a gradual reduction in saturated fats, sugars and salt/sodium and trans fats in foods and beverages. This aims to avoid excessive consumption by consumers and improve the nutrient content of the food as needed [22]. From this perspective, Morocco initiated a national multisectoral strategy for the prevention and control of NCDs 2019-2029 setting a target of reducing 10% of the population's salt/sodium intake [23].

The present study aimed to assess, on one hand, the effect of a salt reduction strategy in baked bread without affecting its textual and structural characteristics. On the other hand, we intended obtaining products of high sensory quality and assessing the effect of the reduction of the acceptability of Moroccan baked bread.

In sum, three primary objectives were fixed: (1) assess the overall appreciation of bread taste; (2) follow the intensity of salty taste of bread salt-reduced; and (3) evaluate the willingness to purchase salt reduced bread.

2. SUBJECTS AND METHODS

2.1. Study design and subjects

2.1.1. Recruitment

This is a cross-sectional and simple-blind experimental study that was conducted in the region of Rabat-Salé-Kénitra at households, at the level of Ibn Tofail University and the Joint unit of Nutrition and Food Research.

2.1.2. Inclusion criteria

All the participants included in the study were aged 15 years and over, supposed to be healthy and regular bread consumers

2.1.3. Exclusion criteria

Subjects under the age of 15 years, pregnant women, individuals with taste disorders, being sick or under medical treatment, were excluded. Only healthy subjects were recruited to determine the level of salt reduction in bakery bread.

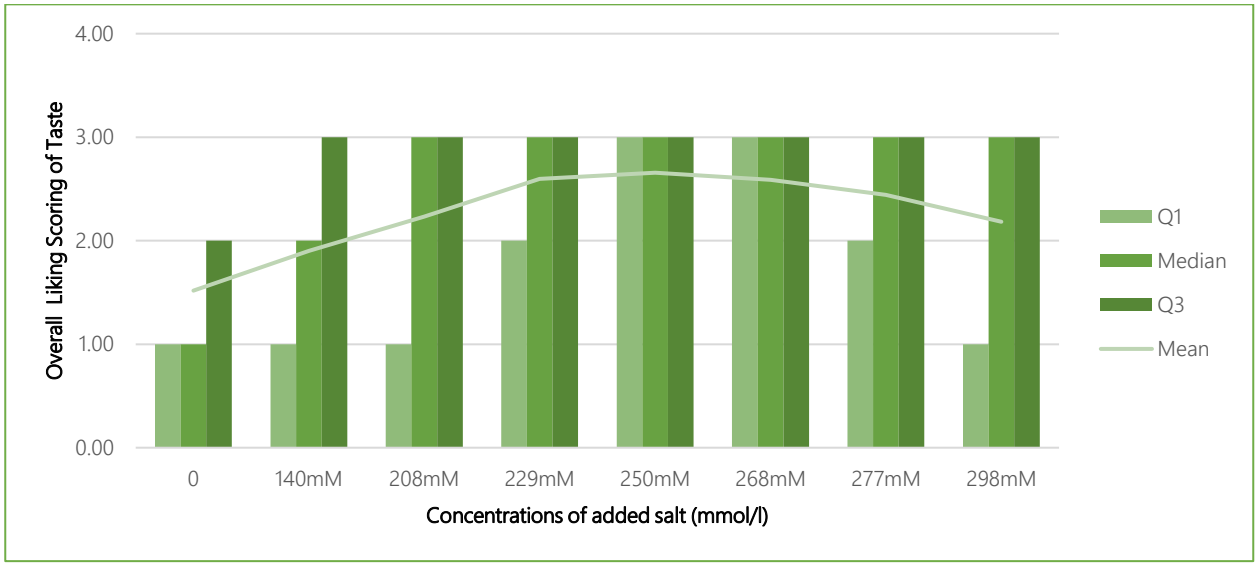

Figure 1: Participants distribution according to quartiles of their overall liking score of bread with varying salt concentration (n = 201) (Q1 = Quartile 1and Q3 = Quartile 3)

2.1.4. Study population

The sensory panel consisted on 201 volunteers classified on four age categories: 15-29, 30-44, 45-59 and over 60 [24]. Informed consent from participants was obtained before starting activities. After consent, participants respond to a short questionnaire about sociodemographic data (age and gender).

The study population was divided into 104 males and 97 Females. General information on age, gender, height, and weight was requested from the participants [24] who were asked to complete a questionnaire and sign an agreement. Different measurements and tests were performed.

2.1.5. Anthropometric measurements

Anthropometric measurements were performed according to the standard WHO procedures [25].

Body Weight

Body Weight was measured at the nearest of 0.1 kg using a mechanical scale (Seca Gmbh, Germany).

The body weight measurement was conducted by ensuring that the subject was wearing less clothing and without shoes. Every subject was placed in the middle of the scale, in the upright position, feet slightly apart and motionless until the body weight value was displayed.

Height

Height was measured at the nearest 0.1 cm using a stadiometer (formerly 130 Shorr Productions, LLC, USA). The participants were barefoot, in a standing position and lightly dressed. BMI was calculated as weight in kilograms by the square of the height in meter (kg / m^2).

2.2. Bread-making procedure

2.2.1. Materials

Wheat flour, durum wheat flour, fresh yeast, NaCl sodium chloride, and water.

2.2.2. Preparation of standard bread

In a kneading machine, 500 g of wheat flour, 500 g of durum wheat flour, 10 g of fresh yeast, 17, 42 g of NaCl and 1000 ml of warm water were mixed for 10 min at 180 rpm and then for 5 min at 250 rpm. The dough was manually kneaded 5 min for an optimal development and then formed into small balls, each weighing 100g. After a rest period of 20 min at 37°C, the dough balls were pressed to a thickness of 3 cm, and left to rest for 20 min at 37°C then pricked and baked for 4 min at 270°C.

This standard recipe brought 1.74 g sodium/100 g final product (fresh weight).

2.2.3. Preparation of bread with reduced-sodium contents

The standard amount of 17.42 g NaCl was reduced to 7%, 10%, 16%, 23%, 30% and 53% [26] to yield 1.62; 1.56; 1.46; 1.34; 1.21 0.81 g of sodium /100g final product respectively. The same protocol was followed to prepare the different series of salt-reduced bread. The bread was cooled and frozen for later tests.

Table 1: Amount of salt added to bread

Salt contents (g)	0	8.19	12.19	13.41	14.63	15.68	16.20	17.42
Concentrations (mmol/L)	0	140	208	229	250	268	277	298
Percentage of salt reduction (%)	100%	53%	30%	23%	16%	10%	7%	0%

Table 2: Participants distribution according to their overall liking score of different bread salt reduction (n = 201)

	Percentage of reduction								p-value
Liking score	100%	53%	30%	23%	16%	10%	7%	0%	p<0,000
I dislike	70.1	48.3	28.4	12.9	12.4	16.9	22.4	35.3	
I neither like nor dislike	8.0	13.4	19.9	14.1	9.5	7.5	10.9	10.9	
I like	21.9	38.3	51.7	72.6	78.1	75.6	66.7	53.7	

Results are presented as percentages of the total population by each salt reduction. *p*-value was calculated using Freidman's Chi2test = 315.059, test significance was set at a p-value < 0.05

Table 3: Participants distribution according to their JAR score of different bread salt reduction (n = 201)

	Percentage of reduction								p-value
JAR scores	100%	53%	30%	23%	16%	10%	7%	0%	p<0,000
A little salty	92.0	76.6	62.2	45.3	21.9	8.5	9.5	10.0	
Just about right	7.0	22.9	32.8	74.8	56.2	50.2	35.3	14.4	
Too salty	1.0	0.5	5.0	7.0	21.9	41.3	55.2	75.6	

Results are presented as percentages of the total population by each salt reduction. *p*-value was calculated using Freidman's Chi2 test = 853,789, test significance was set at a p-value < 0.05

2.2.4.Sensory analysis

Participants were requested to taste the bread and to evaluate their overall liking based on the taste scale that consisted of 9 hedonic scale points (From 1 = I extremely dislike to 9 = I extremely like). The 9 points were grouped into 3 categories: 1 = I dislike, 2 = I neither like or dislike and 3 = I like. Saltiness intensity was assessed using the JAR (Just About Right) on a 9-points scale (1-4 = not salty enough, 5 = just about right, 6-9 = too much salty). Purchase intent was scored on a 5-point scale (1=would definitely not buy, 5 = would definitely buy) [27].

2.2.5. Two-AFC Alternative Forced Choice tests

The evaluation of saltiness perception in bread was performed as described for bread by Pflaum *et al.* [28]. The frozen bread was thawed, brought to room temperature (22°C) and cut into pieces of 3 g. Two three-digit random number encrypted sensory flasks containing different bread samples (3 g each) were presented to each participant in an AB or BA presentation design randomized over the subjects (2 alternative forced-choice). The participants were instructed to rinse their mouths with mineral water during the break (one minute), between tasting the eight bread samples, to eliminate interference carryover effects. Two sensory flasks with different bread samples were presented to each panelist in a randomized order [26].

2.3. Statistical analysis

The statistical analysis was performed using SPSS (version 21). Data were analyzed using ANOVA at p-value ≤0.05. Mean values were compared using the FISHER test. Scores' distribution frequency was calculated for the three used scales (taste scale, JAR scale, and purchase intent scale). The percentage (%) of scores in each category was calculated and Freidman's Chi^2 test was applied to assess the significance between the different categories.

3. RESULTS

3.1. Overall Liking

Table 1 displays the added amounts of salt to the experimental bread and Table 2 summarizes the taste scale results. 78.1% of the participants presented the highest liking taste score for bread with 250 mM corresponding to -16% decrease of added salt with a significant difference (p<0.000) between different reduction regarding perception. The taste of bread with 268 mM, corresponding to -10% of the added salt, was liked by 75.6% of the participants. These findings were confirmed by the first quartile evolution (Q1) of hedonic test scores by participants presented on figure 1. The highest Q1 taste score was obtained for the concentration of 250 mM (Q1=3). Q3 for 250 mmol/L was equal to that for 268mM, 277 mmol/L, and 298 mmol/L indicating that liking bread with salt reduction at 16% was equal to 0%.

Table 4: Participants distribution according to their purchase intent scoring of different bread salt reduction (n = 201)

	Percentage of reduction								p-value
Purchase intent scale	100%	53%	30%	23%	16%	10%	7%	0%	
1. I would definitely not buy	50.2	22.9	5.5	2.0	6.5	7.0	10.0	19.4	p<0,000
2. I would probably not buy	15.9	26.4	13.4	2.5	5.0	7.0	10.0	16.4	
3. I don't know	9.0	12.4	33.8	22.4	10.4	10.4	17.9	16.4	
4. I would probably buy	16.9	22.9	26.9	41.3	36.3	27.9	28.9	25.4	
5. I would definitely buy	8.0	15.4	20.4	31.8	41.8	47.8	33.3	22.4	

Results are presented as percentages of the total population by each salt reduction. *p*-value was calculated using Freidman's Chi2test = 361,213, test significance was set at a p-value < 0.05

Table 5: Participants distribution of their overall liking score of each salt reduction according to sex

Salt reduction (%)	Liking score	Total	Women *(n=104)*	Men (n=97)	p-value
100	I dislike	141	66 (46.8%)	75 (53.2%)	0.634
	I neither like nor dislike	16	9 (56.3%)	7 (43.8%)	
	I like	44	22 (50.0%)	22 (50.0%)	
53	I dislike	97	22 (43.3%)	55 (56.7%)	0.391
	I neither like nor dislike	27	17 (63.0%)	10 (37.0%)	
	I like	77	38 (49.4%)	39 (50.6%)	
30	I dislike	57	20 (35.1%)	37 (64.9%)	0.019
	I neither like nor dislike	40	20 (50.0%)	20 (50.0%)	
	I like	104	57 (54.8%)	47 (45.2%)	
23	I dislike	26	10 (38.5%)	16 (61.5%)	0.070
	I neither like nor dislike	29	10 (34.5%)	19 (65.5%)	
	I like	146	77 (52.7%)	69 (47.3%)	
16	I dislike	25	11 (44.0%)	14 (56.0%)	0.381
	I neither like nor dislike	19	7 (36.8%)	12 (63.2%)	
	I like	157	79 (50.3%)	78 (49.7%)	
10	I dislike	34	15 (44.1%)	19 (55.9%)	0.846
	I neither like nor dislike	15	9 (60.0%)	6 (40.0%)	
	I like	152	73 (48.0%)	79 (52.0%)	
7	I dislike	45	24 (53.3%)	21 (46.7%)	0.241
	I neither like nor dislike	22	13 (59.1%)	9 (40.9%)	
	I like	134	60 (44.8%)	74 (55.2%)	
0	I dislike	71	34 (47.9%)	37 (52.1%)	0.897
	I neither like nor dislike	22	12 (54.5%)	10 (45.5%)	
	I like	108	51 (47.2%)	57 (52.8%)	

Results are presented as effective (percentage). *p*-values were calculated by one-way ANOVA for means

3.2. Saltiness Liking

Participants' distribution, according to their JAR score of bread with varying salt concentration, is reported in table 3. Results show that 76.6% of participants confirmed that bread with a concentration of 298 mmol/L salt/l (0% reduction) was too salty, while 92% declared that 0 mmol/L (100% reduction) was not salty enough. Reductions of 16% (250 mmol/L) and of 10% (268mmol/L) were considered as "Just About Right" by 56.2% and 50.2% respectively. A significant (p<0.000) difference was reported between different reduction according to JAR.

Table 6: Participants distribution of their overall liking score of each salt reduction according to age groups

Salt reduction (%)	Liking score	Total	Age groups (year) 15-29 (140)	29-43 (31)	43-58 (19)	>58 (11)	p-value
100	I dislike	141	107 (75,9%)	22 (15,6%)	8 (5,7%)	4 (2,8%)	0.001
	I neither like nor dislike	16	9 (56,3%)	3 (18,8%)	3 (18,8%)	1 (6,3%)	
	I like	44	24 (54,5%)	6 (13,6%)	8 (18,2%)	6 (13,6%)	
53	I dislike	97	79 (81,4%)	14 (14,4%)	3 (3,1%)	1 (1,0%)	0.000
	I neither like nor dislike	27	17 (63,0%)	6 (22,2%)	3 (11,1%)	1 (3,7%)	
	I like	77	44 (57,1%)	11 (14,3%)	13 (16,9%)	9 (11,7%)	
30	I dislike	57	48 (84,2%)	7 (12,3%)	2 (3,5%)	0 (0,0%)	0.002
	I neither like nor dislike	40	29 (72,5%)	8 (20,0%)	2 (5,0%)	1 (2,5%)	
	I like	104	63 (60,6%)	16 (15,4%)	15 (14,4%)	10 (9,6%)	
23	I dislike	26	25 (96,2%)	0 (0,0%)	1 (3,8%)	0 (0,0%)	0.036
	I neither like nor dislike	29	19 (65,5%)	7 (24,1%)	3 (10,3%)	0 (0,0%)	
	I like	146	96 (65,8%)	24 (16,4%)	15 (10,3%)	11 (7,5%)	
16	I dislike	25	21 (84,0%)	2 (8,0%)	1 (4,0%)	1 (4,0%)	0.255
	I neither like nor dislike	19	15 (78,9%)	3 (15,8%)	1 (5,3%)	0 (0,0%)	
	I like	157	104 (66,2%)	26 (16,6%)	17 (10,8%)	10 (6,4%)	
10	I dislike	34	30 (88,2%)	1 (2,9%)	2 (5,9%)	1 (2,9%)	0.162
	I neither like nor dislike	15	9 (60,0%)	5 (33,3%)	1 (6,7%)	0 (0,0%)	
	I like	152	101 (66,4%)	25 (16,4%)	16 (10,5%)	10 (6,6%)	
7	I dislike	45	32 (71,1%)	7 (15,6%)	4 (8,9%)	2 (4,4%)	0.737
	I neither like nor dislike	22	20 (90,9%)	1 (4,5%)	0 (0,0%)	1 (4,5%)	
	I like	134	88 (65,7%)	23 (17,2%)	15 (11,2%)	8 (6,0%)	
0	I dislike	71	48 (67,6%)	9 (12,7%)	7 (9,9%)	7 (9,9%)	0.221
	I neither like nor dislike	22	11 (50,0%)	7 (31,8%)	3 (13,6%)	1 (4,5%)	
	I like	108	81 (75,0%)	15 (13,9%)	9 (8,3%)	3 (2,8%)	

Results are presented as effective (percentage). *p*-values were calculated by one-way ANOVA for means

3.3. Purchase Intent

Table 4 shows that the best average score of purchase intent was obtained for salt concentration of 268mM. Less than one third of participants (22.4%) confirmed their intention to purchase bread with 298mM salt concentration (0% reduction) besides 47.8% and 41.8% for bread with salt concentration of 268 mM and 250mM respectively. A significant difference ($p<0.000$) between salt reductions based on purchase intent score was recorded.

In table 5, are presented, the results of participant's distribution of their overall liking score of each salt reduction according to gender. The chi-square test shows a significant difference ($p\leq0.05$) between overall appreciation of bread taste and gender for the 30% salt reduction with $p=0.019$. Women appreciated the taste of bread, reduced to this salt content, much more than men with a percentage of 54.8% *versus* 45.2%.

Data about the distribution of participants according to different age categories, for their overall liking, is reported in table 6. For all concentrations, the large size was found in the age category of subjects between 15 and 29. A significant ($p<0.05$) difference was recorded between different age categories from 100% to 23% reductions. Results showed that young subjects presenting the age category between 15 and 29 years old appreciate low-salt bread without affecting their acceptability.

4. DISCUSSION

In terms of ensuring food safety and improving nutritional practices, the WHO has set a global plan of action for the control of NCDs considering a reduction of 30 % of salt intake by 2025 [28]. It should be reminded that the current study constitutes a part of the national multi-sectoral strategy 2019-2029 [23] for the prevention and the control of NCDs. The primary emphasis is to study the feasibility of

salt reduction in bakery bread and acceptability among Moroccan subjects aged from 15 to over 60 years old.

Our results showed that 70.1% of the tasters disliked the taste of bread with 0 mmol/L (corresponding to 0% of added salt), while the taste of bread at a concentration of 250 mmol/L (16% of salt reduction) was appreciated. 78.1% of the population answered by "I like it a lot" against 12.4% who answered "I hate". 75.6%, 72.6% of participants liked the taste of bread at 10% and 23% respectively.

Compared with other studies, these findings were also reported by Miller & Hoseney [29], who showed that salt-free baked bread possesses a tasteless taste. Indeed, in the absence of salt, bread was described as "yeasty," "sour," or "acidic" and having "sourdough" type characteristics [30]. In terms of reduction, it can be judged that around 23%, salt reduction is undetectable by consumers which has been well reported by Girgis *et al.* who demonstrated that the salt content of bread can be reduced by 25% with no detection [31]. Another study showed that a salt reduction of 10 to 20% in whole bread does not affect the taste of the bread and could not be detected by tasters [32]. In Germany, an identical study was performed by Mueller *et al.* [26] who adopted the same strategy of salt reduction with the same levels (from 7% to 53%) in pizza crust showed that 7% and 10% sodium reduction were not significantly discriminable from the standard amount, while 16% and 23% reduction tasted significantly less salty. Moreover, Pasqualone *et al.* [33], showed that would be possible to decrease the salt amount of bread formulation to 15 g/kg without significantly affecting consumer's appreciation. 15g/kg corresponds to 10% of salt reduction in our study that agrees with our results.

According to gender, our results underlined a significant difference between overall appreciation of bread taste and this parameter for 30% salt reduction. Women appreciated the taste of bread reduced salt content much more than men. This disagrees with Weiffenbach *et al.* [34], who found no differences in flavors perceptions according to gender.

Concerning age, young participants responded positively to 16% and 10% of salt reduction. However, further age categories responded to the concentration of 208 mM (30% salt reduction). In this context, some authors showed that cultural influences, age, gender and taste phenotype present an effect on taste appreciation [35,36]. Another study, conducted in Australia, demonstrated this interaction between age and salt perception too. 79% of middle-aged women, especially the age category (22-62 years), consumed an average of 25 bread slices a week up to six consecutive weeks of bread with a 5% discount. Women aged 45-60 enjoyed the taste of bread just in the fourth week of testing. The study concludes that 25% of sodium reduction can be delivered over a short period of time while maintaining consumer acceptance [31].

For the salt intensity in bread, 56.2% of our study population responded by "just about right", while 21.9% of them consider it less salty at the concentration of 16%. Compared to their purchase willingness to each bread type, 21.8% of the population agree to purchase bread corresponding to the concentration of 268 mM (23% salt reduction), and 41.8% agree purchasing bread at 16% of salt reduction, whereas, 20.4% at 30% of salt reduction. La Croix *et al.* [37] found that reducing sodium levels in bread up to 30% did not affect consumer liking or purchase intent of the products.

Additionally, it was shown that a 50% reduction in bread salt did not reduce bread consumption or affect the choice of sandwich fillings [38]. Bertino *et al.* [39] reported that, when subjects ingest solid or liquid foods with salt reduction by 25%, they appreciate the taste foods just after 2 months against the original ones that become too salty in the long term.

In summary, our results highlight the long-term feasibility of a substantial reduction in daily salt intake. In addition, other studies showed that when the reduction in salt content of food is performed gradually, the intervention goes unnoticed for the majority of people. A study, carried out by the health monitoring institute, showed that people consuming large amounts of salt have a caloric intake increase, appetite and thirst [40]. High salt consumption is frequently accompanied by an increase in food consumption, snacking and sweet drinks primarily among young consumers who may become addicted to and, as a consequence, could develop obesity. Indeed, several epidemiological studies have found that high salt intake is correlated with a marked increase in the risk of developing NCDs such as hypertension, cardiovascular diseases, cancer, diabetes and other pathologies which are the leading cause of worldwide mortality [20].

In Morocco, there has been a rapid increase in the prevalence of obesity within the population [9,41]. According to STEPS, a recent survey conducted in Morocco, the prevalence of obesity among adults aged more than 18 years old was 20%. Like other countries in the world, this prevalence has increased compared to that of 2000 (13.2%) [42]. Obesity does not concern solely the adult population, however, adolescents aged between 10 and 19 were also considered obese (9%) [20]. Overweight/obesity and high sodium intake are considered as a risk for developing hypertension among adults and children [43]. High blood pressure is affecting 29.3% of Moroccan adults,

this fact has been decreasing slightly between the years 2000 (33.6%) and 2017 (29.3%)[42].

It must be underlined that a healthy diet is a crucial approach for preventing and controlling NCDs. Thus, our study on salt reduction in bread, based on the principle of setting objectives, allows the Moroccan population consuming fewer salt amounts, especially in processed foods and as a result avoiding developing chronic diseases.

Our investigation is comparable to other studies carried out in 19 global countries where salt amounts were reduced in processed foods at 25% depending on the function of the product concerned. Therefore, our study leads to the conclusion that 16% of salt reduction (eq. 14.63 g of salt/kg of flour) in Moroccan bread is a possible target without affecting the taste and the quality of bread consumed by the general population.

5. CONCLUSION

Modest restrictions in salt intake could potentially reduce the risk of morbidity and mortality of cardiovascular diseases. Our results highlight that producing bread containing lower salt levels is technologically feasible. Overall, a 16% reduction of salt did not affect the acceptance of consumers and their willingness to purchase bread. The current study reflects one of the potential strategies for achieving the objective of reducing salt consumption at the level of our population and to decrease the burden of cardiovascular diseases, high blood pressure by reducing its prevalence to 10% by the year 2029.

Some of the potential limitations of this study were that our population presented an unequal distribution according to different age groups with a high effective for participants aged from 15 to 29 years. This may be owing to the encountered difficulty in convincing elderly persons to participate in this survey. While, the final included sample was valid. Nevertheless, further studies will be conducted with the aim of overcoming the present limitations.

Acknowledgments

Special acknowledgments to the respondents of this study, to health workers, local authorities, and to all persons who contributed, directly or indirectly, to the success of this work.

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Cite this article as: Guennoun Y, Bouziani A, Bajit H, El Berri H, Elammari L, Benaich S, Barkat A, Benkirane H, and Aguenaou H. Acceptance of salt reduction in bakery bread among Moroccan consumers. *Nor. Afr. J. Food Nutr. Res.* July - December (2019);03(06):219-28. https://doi.org/10.5281/zenodo.3592037